Characterization of substrate binding and enzymatic removal of 3-methyladenine lesion from genomic DNA with TAG of MDR *A. baumannii*

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ELECTRONIC SUPPLEMENTARY INFORMATION

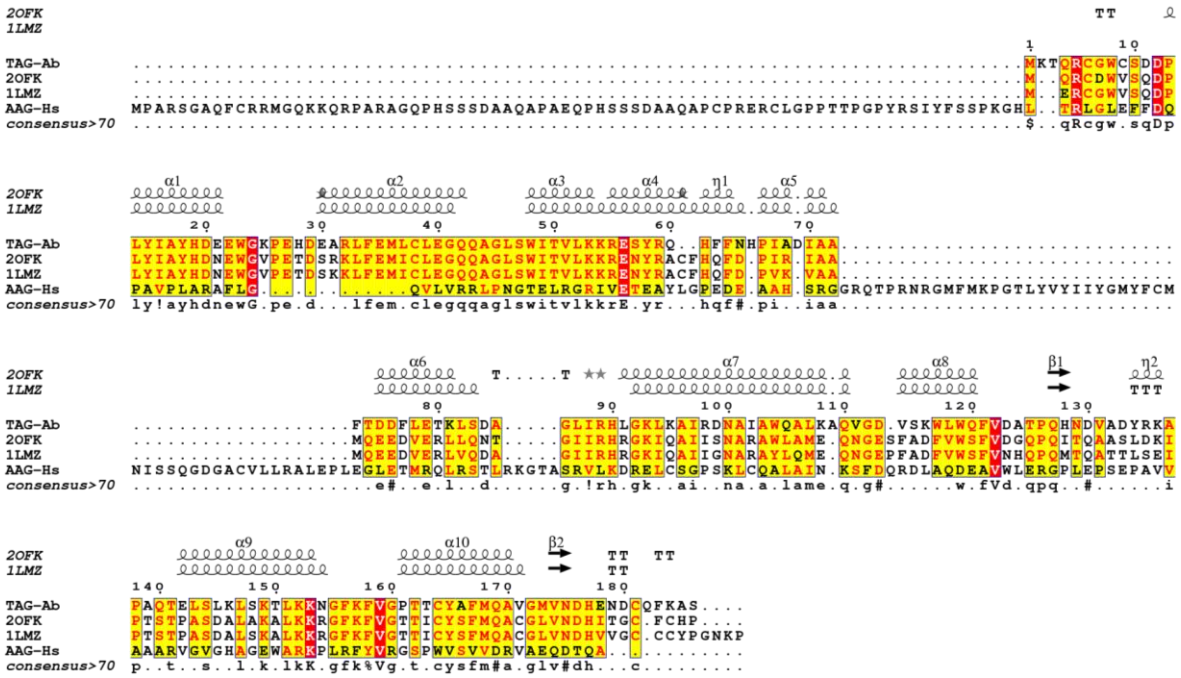


Figure SI-1A. Multiple sequence alignment is generated using the online ESPript 3.0 server. Secondary structural elements are indicated above the sequences; sequences are divided according to similarity: red highlight colour represents strictly conserved residue, yellow represent the residue well conserved, residue having difference are shown in normal. Abbreviations: AAG-Hs, alkyl-DNA glycosylase of *Homo sapiens*).

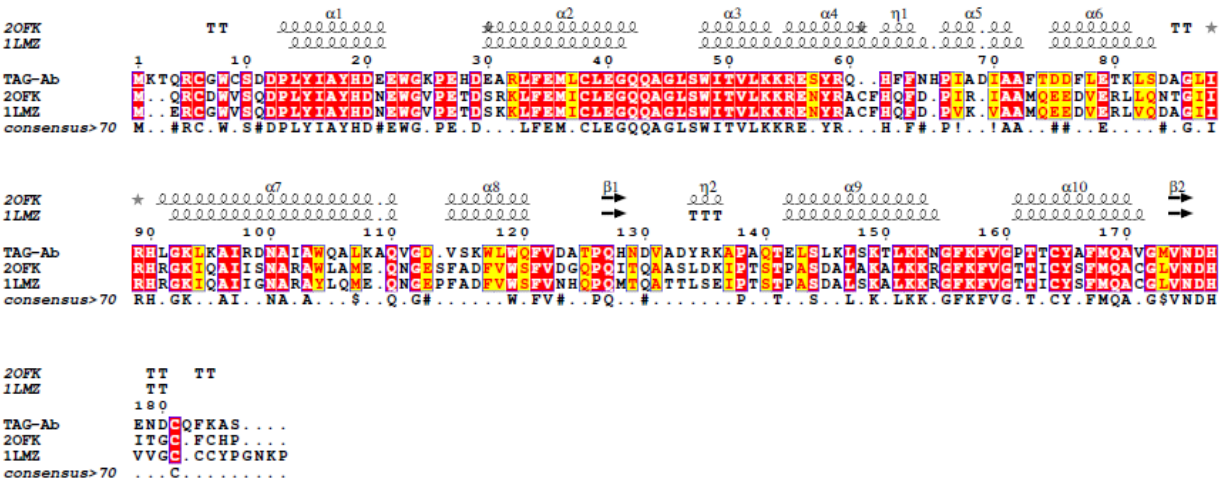


Figure SI-1B. Alignment is generated using ESPript 3.0 server. Secondary structural elements of 2OFK and 1LMZ are indicated above the sequences, with the same colour schemes as in **SI-1A**.

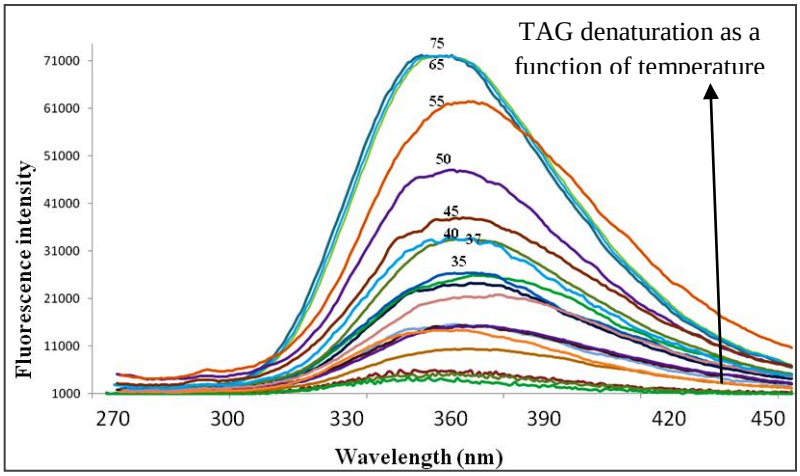
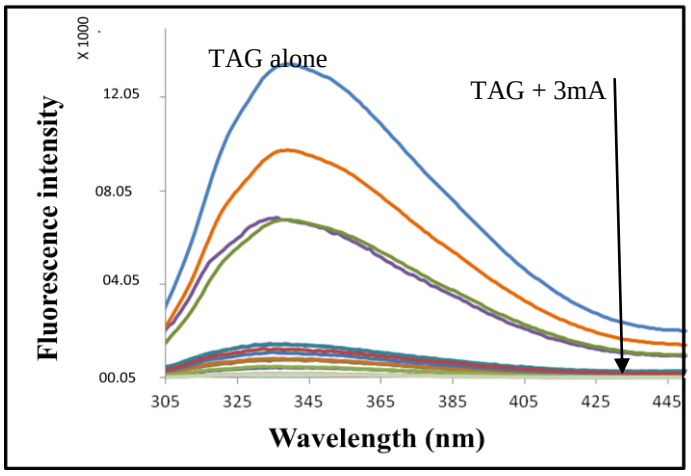


Figure SI-2A. Intrinsic tryptophan fluorescence emission spectrum of TAG alone and after heating (as a function of temperature).

2B



2C

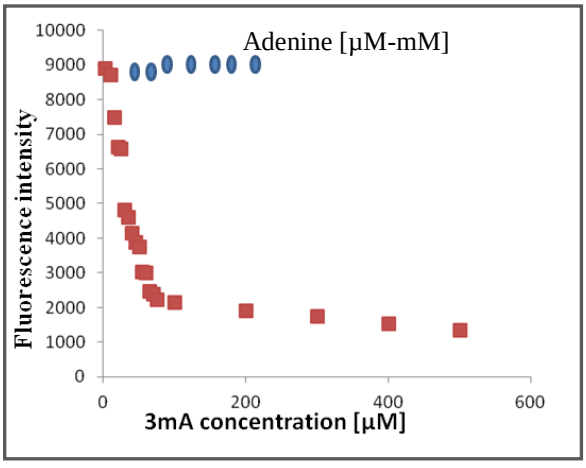


Figure SI-2. **B)** Fluorescence emission spectra of substrate-free TAG before and after titration with 3mA showing the large decrease in intrinsic tryptophan fluorescence. **C)** Fluorescence emission spectra of substrate-free TAG before and after titration with 3mA showing large decrease in intrinsic tryptophan fluorescence in presence of adenine (red square markers) and no change in emission spectra of TAG in titration with adenine (blue round markers).

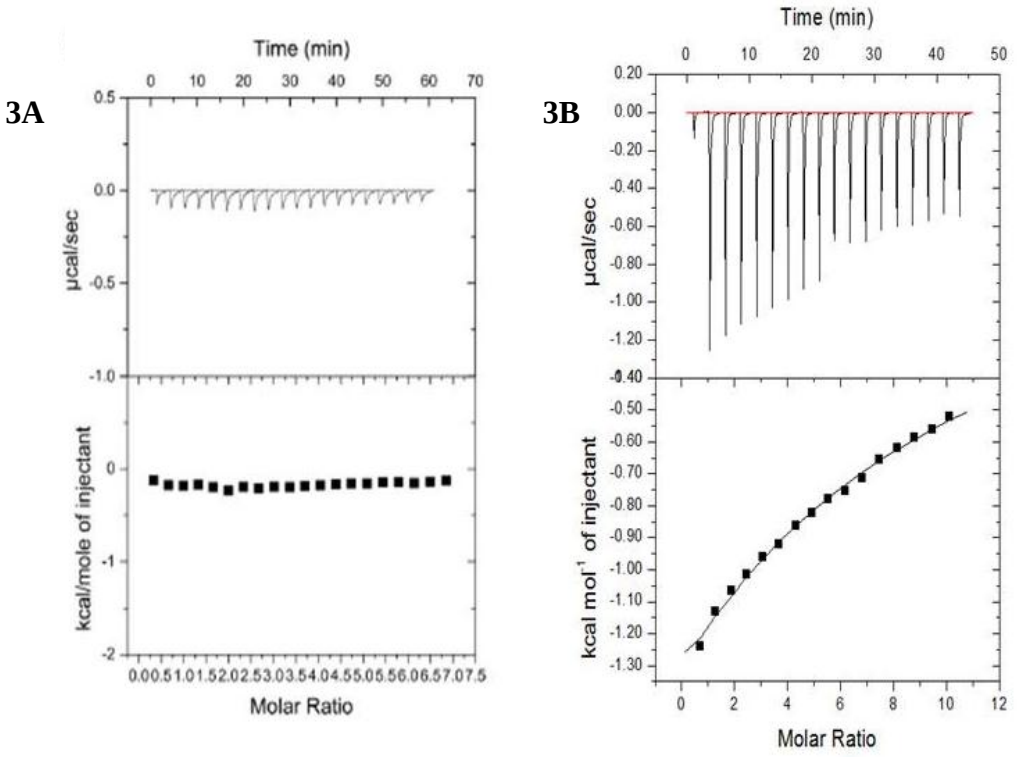


Figure SI-3. **A)** TAG enzyme titration with adenine at 20 ºC, integrated binding isotherm showing no measurable difference in the heat release on titration. **B)** ITC binding isotherm for association of TAG with Zn^{2+}. Upper panels correspond to the total heat exchanged upon injecting the aliquots of substrate. Lower panels show the resultant binding isotherms acquired by integrating the peak areas of each injection.

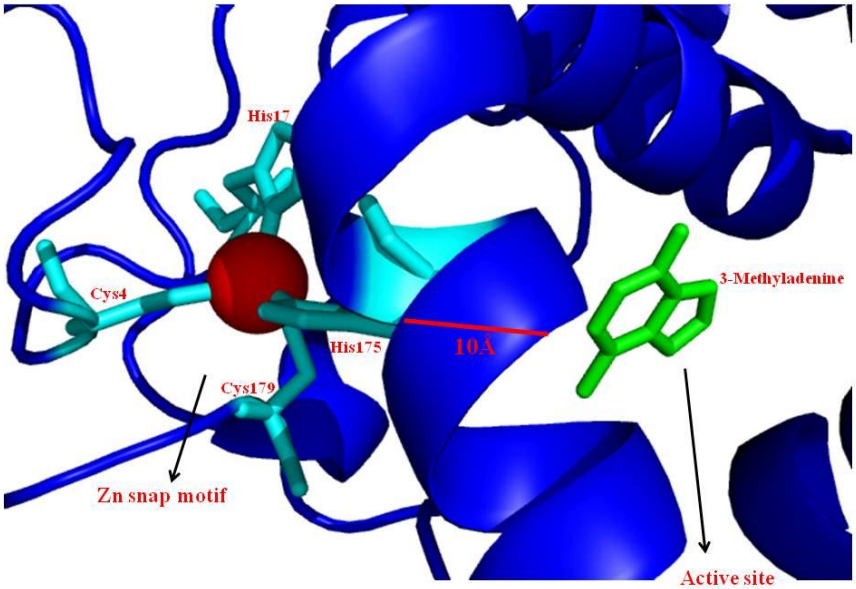


Figure SI-3C. TAG enzyme depicting 3-methyl adenine (green) binding site and zinc binding site, both are located side by side and distance between both site is 10 Å, figure is prepared using pymol plugin.

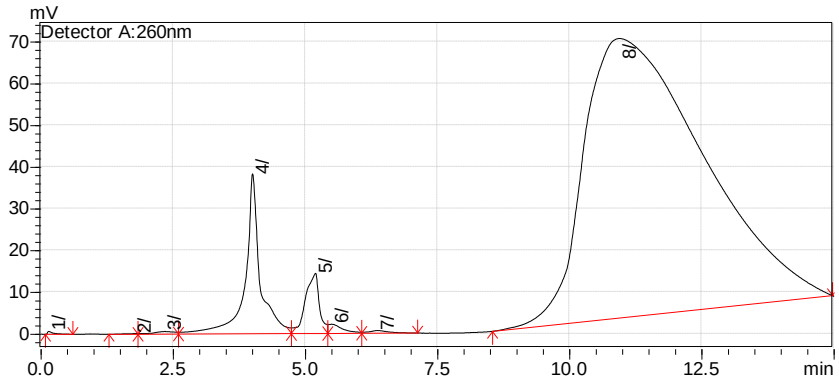


Figure SI-4. RP-HPLC chromatogram of unlabeled methylated nucleobase (3mA), retention times are indicated at each peak. Due to the high polarity of 3mA, an ion-pairing agent (perfluoro-pentanoic acid) was added to improve retention and resolution. TAG was incubated with genomic control DNA at 37 ºC for 20 min, a reaction volume of 20 µL was injected to HPLC column.

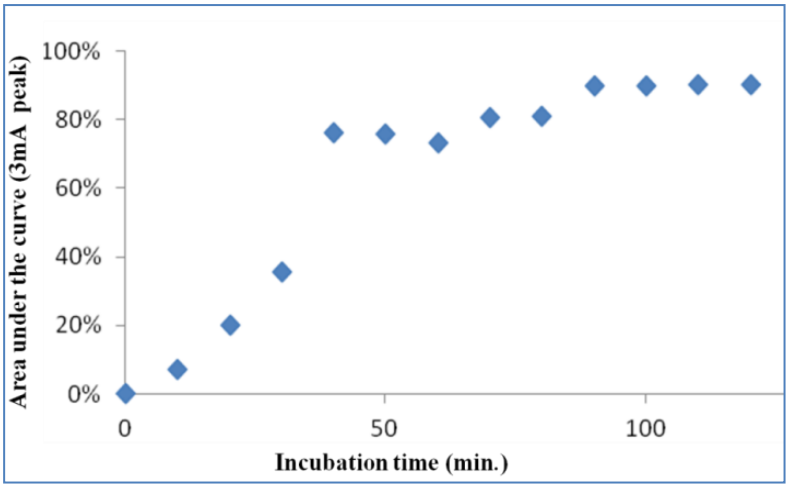


Figure SI-5. 3-Methyladenine excised from the control genomic DNA by the glycosylase activity of the TAG at 4 ºC after incubation time interval 0–120 min. The TAG-excised product is eluted at 12.5 min represents similar retention time as obtained for the pure 3mA.

Table SI-1. Excision of 3mA from the control genomic DNA by the glycosylase activity of the TAG at 4 ºC after incubation time interval 0–120 min. The TAG-excision parameters obtained as a function of varying incubation time are given.

Incubation time (min)	Peak area (3mA)	Peak intensity (3mA)	% of Peak area (3mA)
0	0	0	0
10	207876	2496	7.227
20	554443	3751	20.04
30	5458562	24146	75.59
40	6691799	34819	76.38
50	5720154	31019	75.91
60	3615485	18250	73.16
70	2882102	60885	80.57
80	5202681	37638	81.06
90	10526328	66970	90.04
100	10870199	70827	90.15
110	10871485	67010	90.35
120	10872156	68581	90.48