SUPPLEMENTAL MATERIAL

BIOCHEMICAL AND STRUCTURAL STUDIES ON THE M. TUBERCULOSIS O^6-METHYLGUANINE METHYLTRANSFERASE AND MUTATED VARIANTS

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SUPPLEMENTAL METHODS

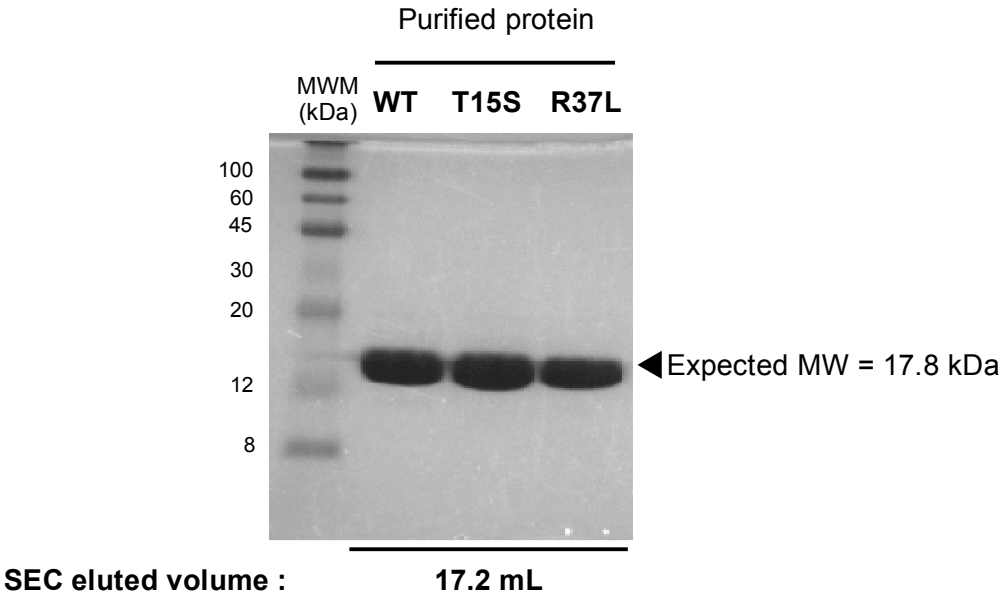
Expression and purification of wild-type MtOGT and point mutants - The three recombinant proteins *Mt*OGT, *Mt*OGT-T15S and *Mt*OGT-R37L were purified by FPLC (Akta Basic Instrument GE Healthcare) from 1 L of the corresponding auto-induced bacterial culture adopting the following workflow. 50 mL of clarified lysate in buffer A [Tris-HCl 20mM pH 7.8 and a commercial protease inhibitor cocktail (Sigma)] were loaded onto a HiTrapQ pre-packed anion exchange column. The retained proteins were then eluted using a linear 0-0.5 M NaCl gradient. Recombinant protein-containing fractions were dialysed against buffer A, applied onto a MonoQ pre-packed column (GE Healthcare) and protein elution was obtained as above. Recombinant protein-containing fractions from the last anion-exchange chromatography step were pooled, desalted by dialysis against buffer A and loaded onto a HiTrap Heparin disposable column (GE Healthcare). Protein elution was obtained by a linear 0-1.0 M NaCl gradient and the eluted fractions containing the recombinant protein were pooled, concentrated to 1 mg/mL by using 10 kDa NMWCO ultrafiltration device (Vivaspin, Vivascience) and loaded onto a Superdex 200 10/300 size exclusion chromatography column (GE Healthcare) using buffer B [20 mM Tris-HCl pH 7.8, 150 mM NaCl] as the mobile phase. Fractions corresponding to the main peak were concentrated to 5 mg/mL by using a 10 kDa NMWCO Vivaspin device. During the entire procedure the recombinant protein was monitored by standard SDS/PAGE analyses (example given in Supplementary Figure 1) and protein concentration was determined by the Bradford assay (Sigma). If not immediately used, the purified proteins were stored in aliquots at -80 °C.

Table S1 - Oligonucleotides used in the present work

***Mt*OGTfwd**[a]	5'-ATATCCATGGTTCACTACCGCACCATCGATAGC-3' (*Nco* I)
***Mt*OGTrev**[a]	5'-AAAGGATCCTCAGTCGAAGAGCGTCAAGTCTG-3' (*Bam* HI)
T15S fwd[b]	5'-CCCATCGGGCCATTA A**G**C CTGGCCGGGCATGGC-3' ACC
T15S rev	5'-GCCATGCCCGGCCAGGCTTAATGGCCCGATGGG-3'
R37L fwd[c]	5'-CGTATGAGCCAAGC C**T**C ACACACTGGACACCC-3 CGC
R37L rev	5'-GGGTGTCCAGTGTGTGAGGCTTGGCTCATACG-3'
UP^{met} [d]	5'-GGACACTGTACGTTAAGGC**G***ATCGAATTAGGATTAA-3'
DOWN[d]	5'-GGTTAATCCTAATTCGATCGCCTTAACGTACAGTGT-3'
A^{+} [e]	5'-**TMR**-GCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA-3'
D^{-} [e]	5'-TAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC-3'

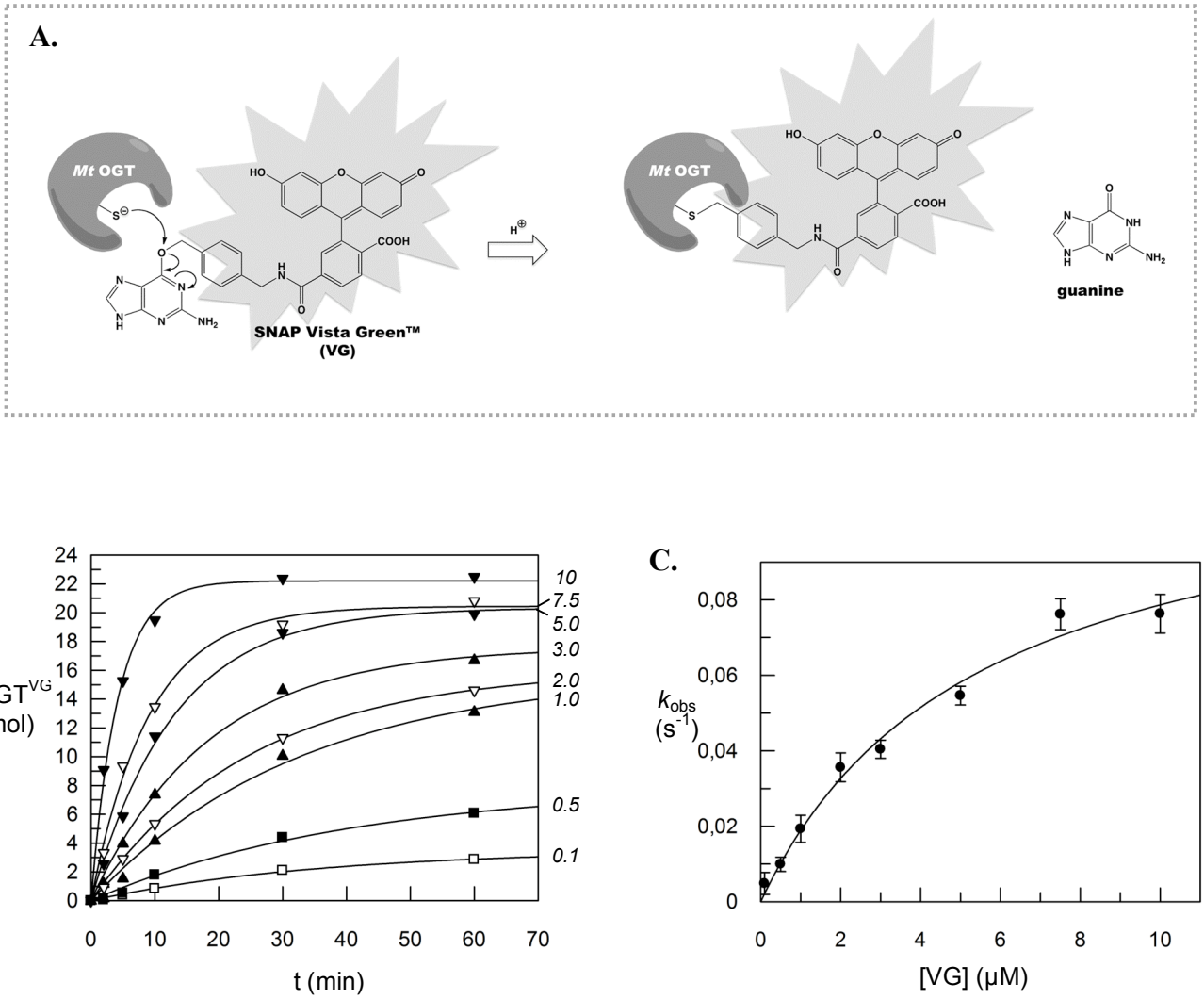
[a] Restriction sites for the endonucleases indicated in brackets are underlined; [b] [c] point-mutation in each oligonucleotide appears in bold; [d] the methylguanine residue in the UP^{met} oligonucleotide is labeled by an asterisk; the ds-DNA^{met} probe used in enzyme kinetics analysis has been prepared by annealing **UP^{met}** and **DOWN** oligonucleotides; [e] "**TMR**" indicates the TAMRA^{TM} fluorescent group; the dsDNA probe used in EMSA analysis has been prepared by annealing **A^{+}** and **D^{-}** oligonucleotides.

Fig. S1



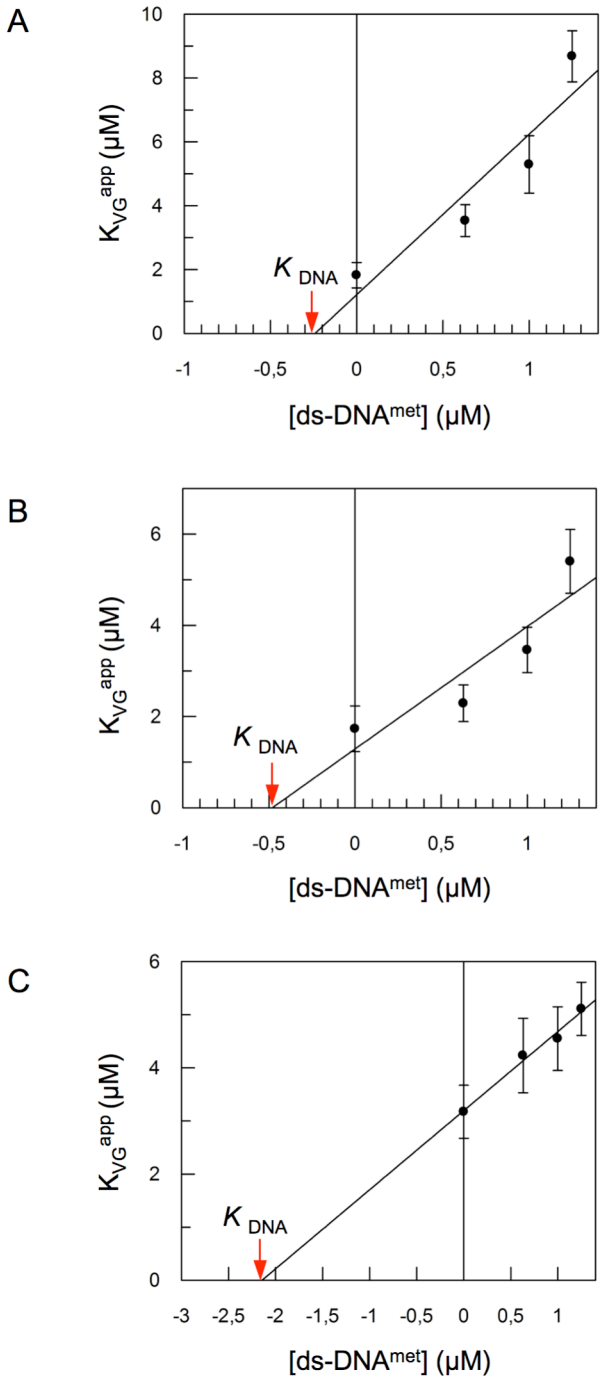
Purification of *Mt*OGT, *Mt*OGT-T15S and *Mt*OGT-R37L - 4 µL of each purified protein (5 mg/mL) were separated using a 15% acrylamide-bisacrylamide gel in SDS-PAGE and visualized by Coomassie brilliant blue staining. The three proteins, independently loaded onto a Superdex 200 10/300 size exclusion chromatography column (GE Healthcare), invariably eluted at V_e=17.2 mL, which corresponds to a MW of 18 kDa, as calculated on the basis of the calibration curve of the column.

Fig. S2



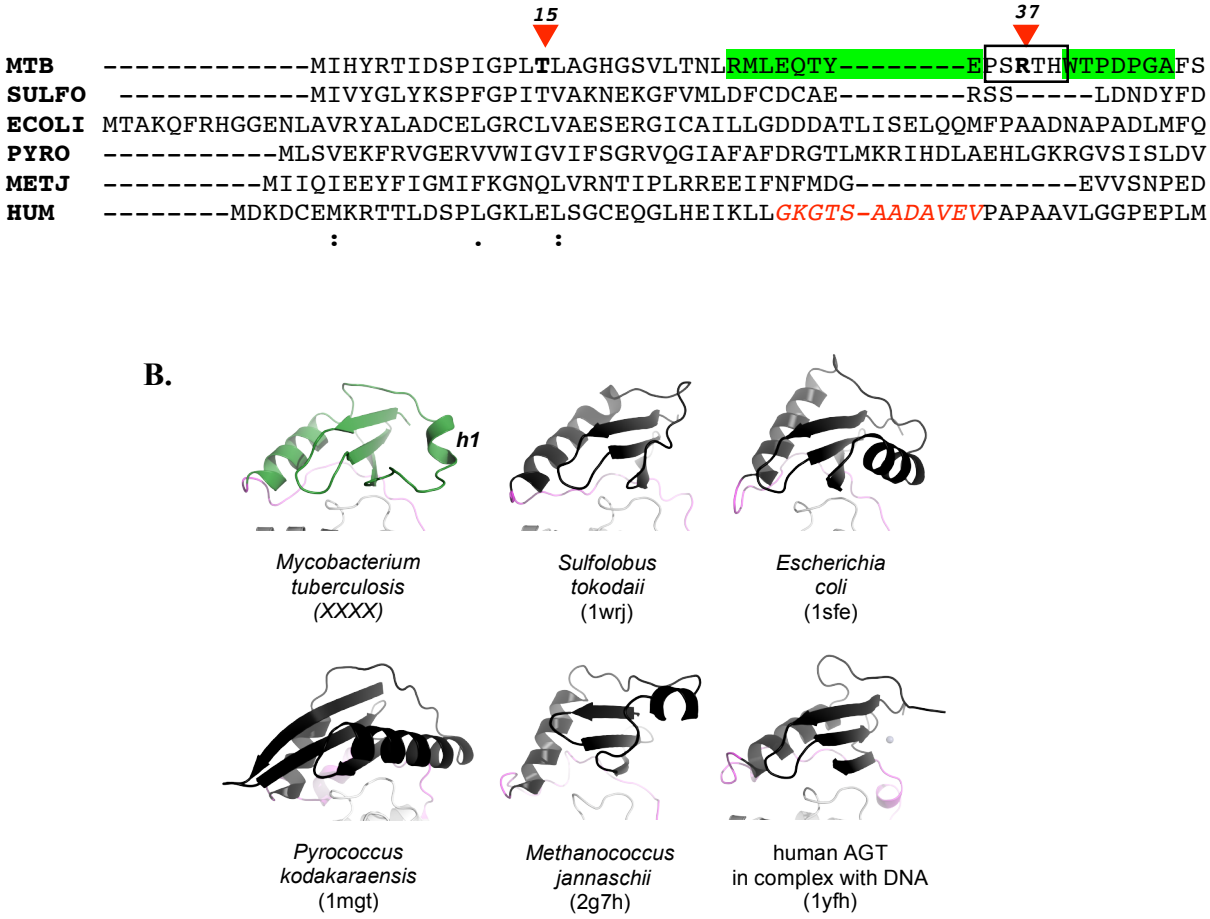
Example of the determination of the kinetic constants of the reaction catalyzed by the recombinant proteins under study. A. Scheme of the assay with the fluorescent substrate/inhibitor SNAP-Vista GreenTM (VG); **B.** Time-course experiments of covalent modification of 5 μM *Mt*OGT incubated with VG in the range of 0.1-10 μM (VG concentration appears at the right of each corresponding curve) and taking aliquots at 0, 2, 5, 10, 30, 60 min. Similar sets of measurements were carried out for both the *Mt*OGT-T15S and *Mt*OGT-R37L mutated proteins (not shown); **C.** Plot of the first-order rate constants for covalent modification of *Mt*OGT (k_{obs}) as a function of SNAP-Vista Green™ (VG) concentration. Rate values obtained in the time course experiments were fitted according to equation **1** (see the "*Materials and Methods*" section of the main text).

Fig. S3



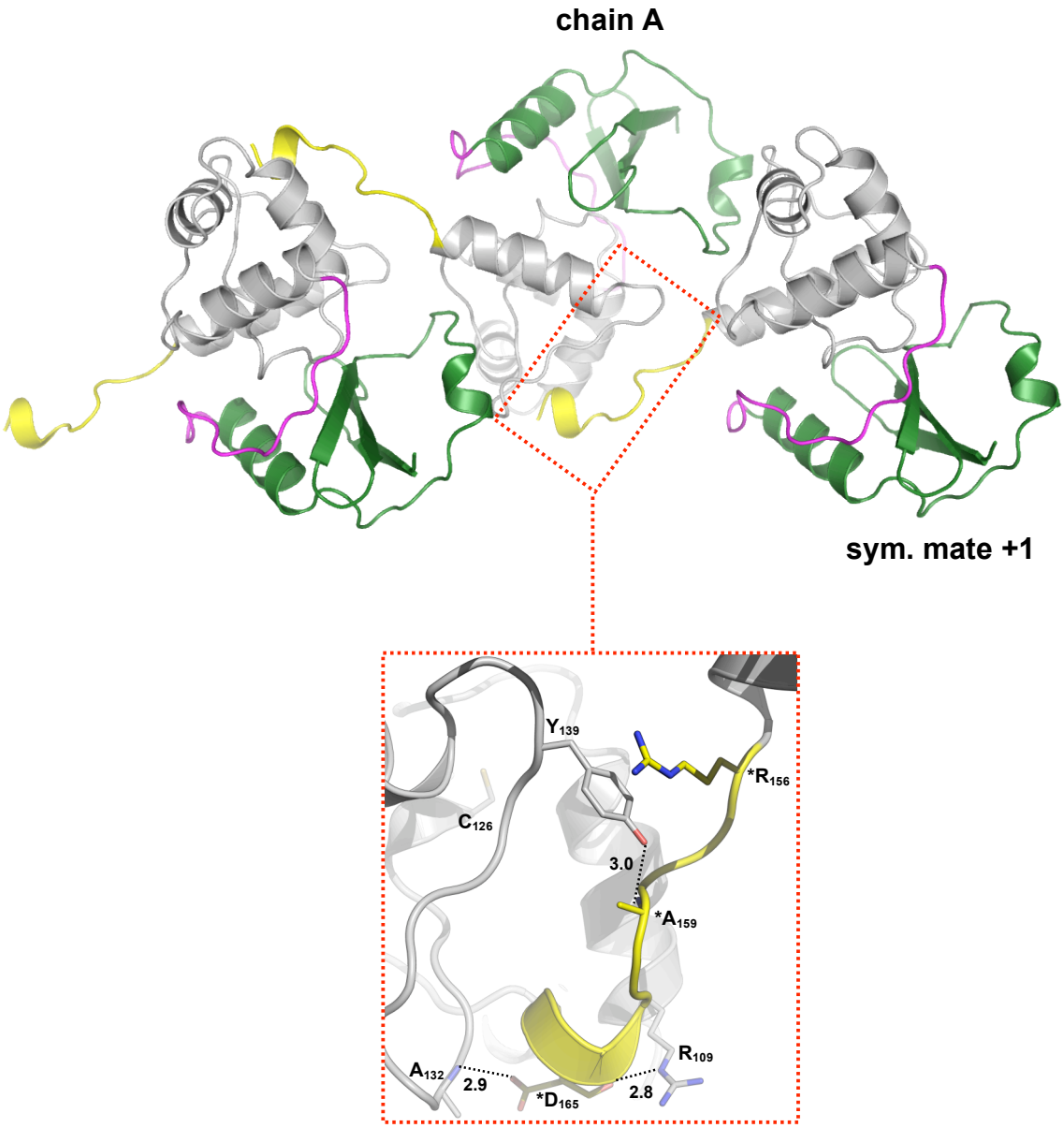
Determination of the DNA dissociation constants of the reaction catalyzed by *Mt*OGT, *Mt*OGT-T15S and *Mt*OGT-R37L - Plots of the variation of K_{VG} of *Mt*OGT (A), *Mt*OGT-T15S (B) and *Mt*OGT-R37L (C) as a function of the ds-DNA^{met} concentration. Data were fitted according to equation **2** (see the "*Materials and Methods*" section of the main text) that allowed the calculation of the dissociation constant of each enzyme for the ds-DNA^{met} ligand (K_{DNA}), indicated by a red arrow in each panel.

Fig. S4



Structural comparison of *Mt*OGT with OGTs from different species - A. The figure shows the primary ClustalW-generated sequence alignment of equivalent moieties of the N-terminal domains of OGTs whose structures have been deposited with the protein data bank. **MTB**: *Mycobacterium tuberculosis*; **SULFO**: *Sulfolobus tokodaii*; **ECOLI**: *Escherichia coli*; **PYRO**: *Pyrococcus kodakaraensis*; **METJ**: *Methanococcus jannaschii*; **HUM**: human AGT. The random-coiled region in *M. tuberculosis* OGT is highlighted in green and residues belonging to the h1 helical turn are boxed. The red arrowheads label the residues mutated in *Mt*OGT-T15S and *Mt*OGT-R37L, respectively. Residues rendered in red in the human sequence are not visible in the human AGT structures. **B.** Cartoons of the indicated structures after optimal superposition to the *Mt*OGT model; each panel shows only the N-terminal domain (depicted in green in *Mt*OGT and in black in all other structures) and part of the inter-domain connecting loop (invariably colored in magenta).

Fig. S5



Crystal packing of *Mt*OGT - Close up view of the interacting regions of the C-terminal domain of one chain (gray) and the tail of its symmetry mate (yellow, residues labeled by an asterisk) as observed in the *Mt*OGT crystal lattice (upper image); only the residues involved in the closest interactions are labeled and shown as sticks; bond distances (Å) are indicated as dotted lines.