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Supporting information for article:

Crystal structures of the closed form of *Mycobacterium tuberculosis* dihydrofolate reductase in complex with dihydrofolate and antifolates

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Table S1 R.m.s.d. (Å) among the structures from different complexes of MtDHFR:NADPH in the closed conformation state.

	CYC	**DIA**	**DHF**	**PMX**	**MTX**	**TMP**
PYR	0.09	0.22	0.42	0.36	0.69	0.65
CYC		0.22	0.41	0.36	0.72	0.66
DIA			0.40	0.30	0.66	0.63
DHF				0.20	0.54	0.60
PMX					0.58	0.60
MTX						0.35

PYR (Pyrimethamine); CYC (Cycloguanil); DIA (Diaverdine); PMX (Pemetrexed); MTX (Methotrexate); TMP (Trimethoprim); The PDB entries for MTX and TMP are 1DF7 and 1DG8, respectively (Li *et al*., 2000).

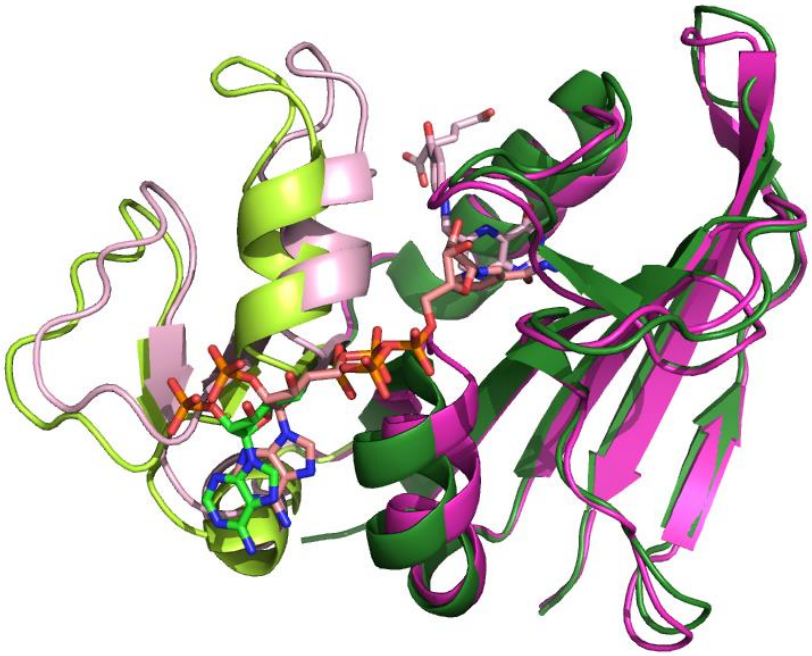

Figure S1 Superposition of closed and open states of MtDHFR. Superposition of closed (MtDHFR:NADPH:DHF) (represented in dark and light magenta) and open conformation of MtDHFR:NADPH (represented in dark and light green) (PDB entry 4KLX) (Dias *et al.*, 2014). The rmsd of the structure superposition is 1.17Å. It is possible to observe the displacement of the adenosine domain (light pink for closed state and light green for open state) between the two structures. on the substrate binding site. In addition in the open state, the nicotinamide moiety of NADPH (green carbon sticks) is not engaged in the active site in contrast to the closed state (NADPH represented with pink carbon sticks).

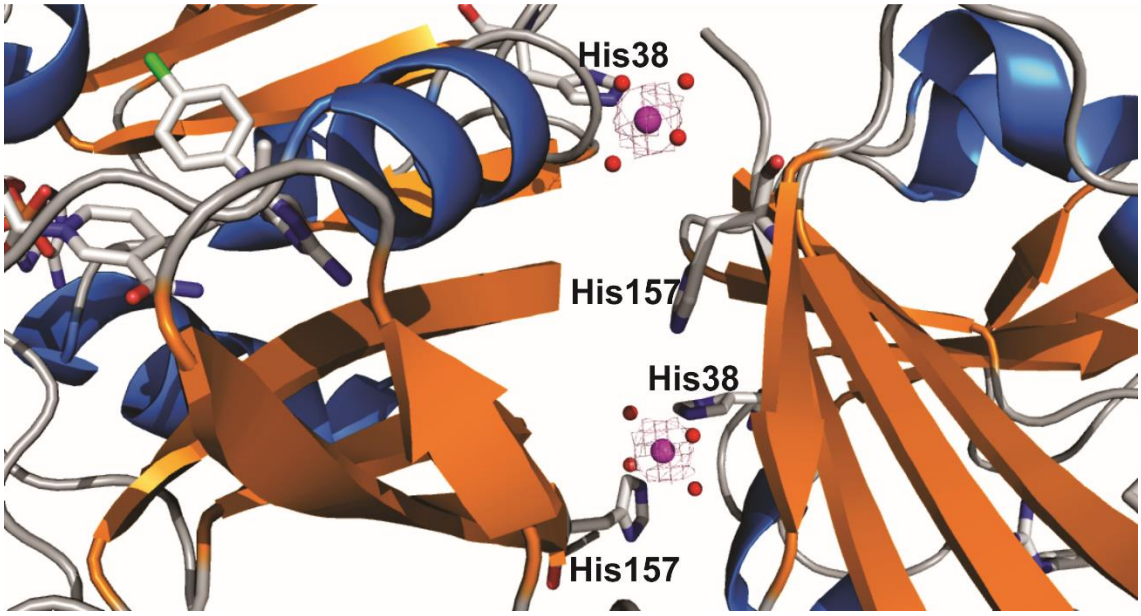

Figure S2 Protomers interface of MtDHFR. In purple is shown the possible cobalt ion. In red is shown water molecules and in sticks the histidines of both protomers. The cobalt ions have a 1σ 2Fo-Fc map electron density contours. However, this coordination by the two pairs of histidines is not observed in all structures and, generally, only one coordination involving both protomers is present since the rotamer of the side chain of His157 can adopt an alternative conformation. In order to evaluate whether the cobalt ion is able to promote the dimerization of the enzyme, we have performed an analytical gel filtration using 20 mM Tris-HCl pH 7.5, 100 mM NaCl, 5% glycerol and 2 mM of cobalt chloride and also an alternative buffer containing 20 mM MOPS (3-(N-morpholino)propanesulfonic acid) pH 7.0, 10 mM cobalt chloride(figure not shown). The chromatogram profile in both conditions indicated that there is no change in the retention volume time in comparison to the buffer in the absence of the ion, strongly suggesting that the cobalt coordination observed in the crystal packing is only a crystallization artifact.

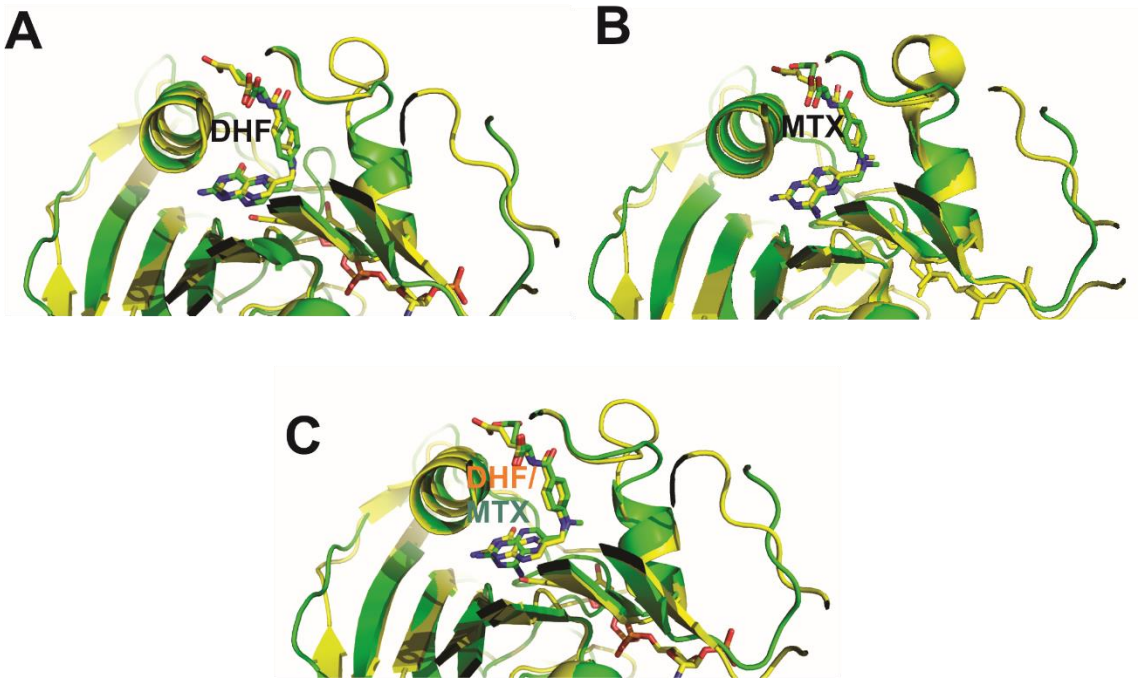

Figure S3 Superposition of MtDHFR (yellow) and EcDHFR (green) in complex with ligands. A) Superposition of holoenzymes in complex with DHF; B) Superposition of holoenzymes in complex with MTX and C) Superposition of MtDHFR:NADPH:DHF and EcDHFR:NADPH:MTX. PDB entries for EcDHFR: 1RF7 for EcDHFR:NADPH:DHF and 1RG7 for EcDHFR:NADPH:MTX complexes (Sawaya & Kraut, 1997).

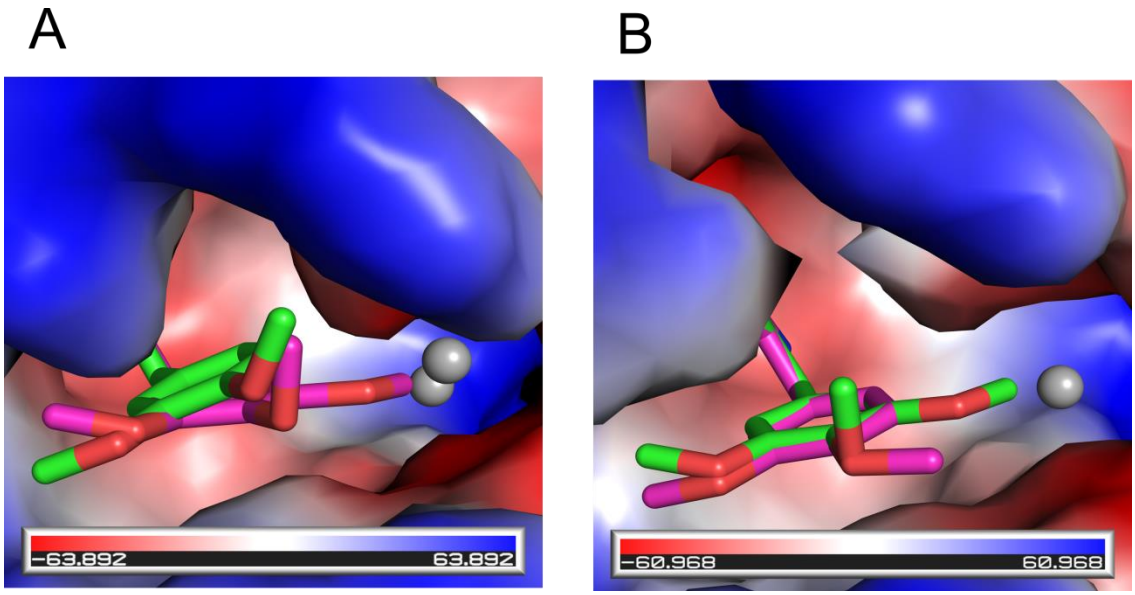

Figure S4 Solvation of the active site of MtDHFR:NADPH:DIA in comparison to MtDHFR:TMP. Conformational changes and solvation determine differences in the thermodynamic profiles of binding of DIA and TMP to MtDHFR. Electrostatic surface potential representation of the antifolate binding pocket of MtDHFR. Panels A and B represent the protomers A and B in the structure of MtDHFR:NADPH:DIA, respectively. DIA is represented in green whereas TMP is in magenta. Water molecules into the hydrophobic binding pocket of MtDHFR:NADPH: DIA are represented as grey spheres. The colour code is red for negative, blue for positive and, white for neutral charges.