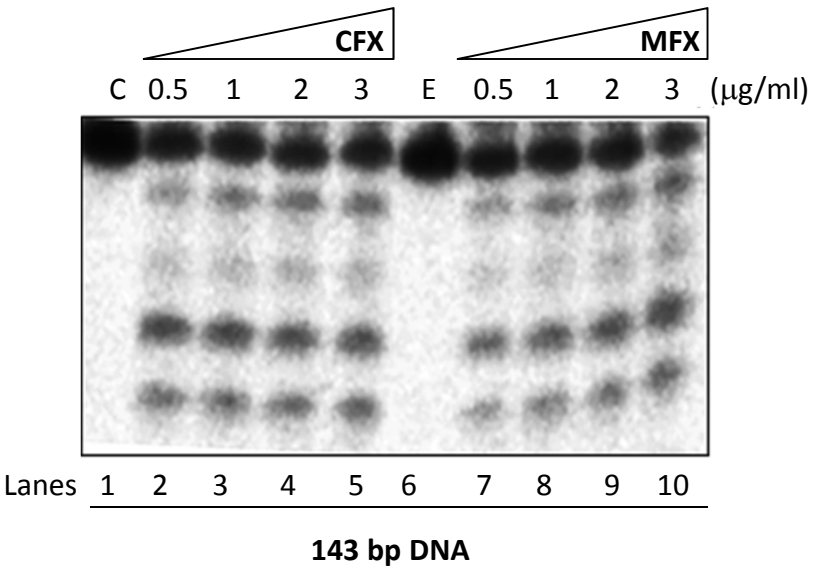

FIG S1. DNA cleavage pattern with *E. coli* gyrase. 50 nM of the enzyme was incubated with 5'- γ ^{32}P labeled 143 bp DNA in presence of varying concentrations (0.5-3 μg/ml) of CFX (lanes 2-5) or MFX (lanes 7-10). C - DNA control (lane 1) and E - enzyme and DNA in absence of any FQs (lane 6). The reactions were carried out as described in the legend of FIG 2.

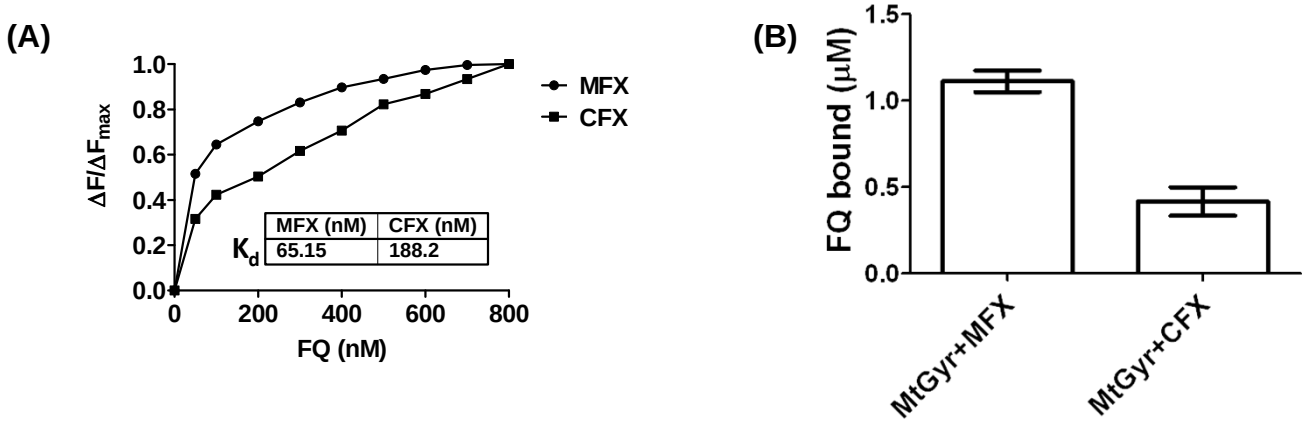

FIG S2. Interaction of *M. tuberculosis* DNA gyrase (MtGyr) with CFX and MFX. **(A)** Binding of FQs to the enzyme monitored by incubation of gyrase (50 nM) in presence of varying drug concentrations (100-800 nM). ΔF- the difference between observed intrinsic fluorescence intensity of the enzyme at any given drug concentration and the intensity in absence of drug, ΔF_{max}- the change in the intensity at infinite concentration of the drug. The K_d was calculated by Hill plot analysis using GraphPad Prism ver. 5. **(B)** Gyrase-FQ binding measured by spin column gel filtration method. 1μM of the enzyme was mixed with 250 μM of either CFX or MFX and incubated for 30 min at 37 °C. The drug-enzyme complex was separated from the free drug molecules by spin column gel filtration (G-25 sephadex). The amount of drug in the complex with gyrase was measured by fluorimetry and the standard plot of the respective drug.

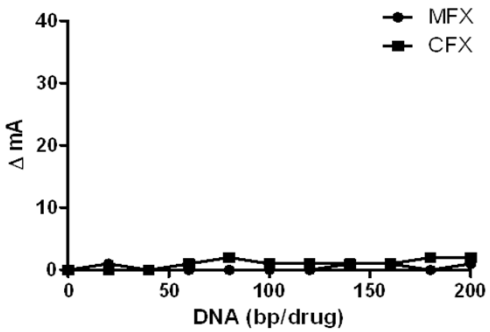

FIG S3. Interaction of CFX and MFX with DNA monitored by fluorescence anisotropy. 10 nM of MFX or CFX was titrated with salmon sperm DNA (bp/drug molecule).

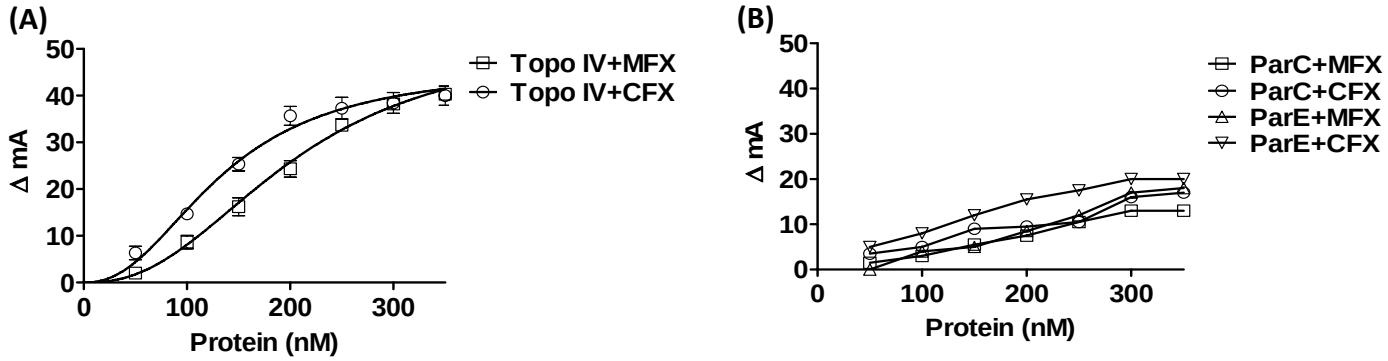

FIG S4. Interaction of CFX and MFX with *E. coli* topo IV and its individual subunits. 10 nM of CFX or MFX was titrated with varying concentrations of **(A)** topo IV holoenzyme and **(B)** individual subunits, ParC and ParE.

FIG S5. Alignment of the amino acid residues from *M. tuberculosis* (Mt)*, M. smegmatis* (Ms) and *E. coli* (Ec) GyrA. The serine 83 and aspartate 87 in *E. coli* GyrA correspond to alanine 90 and aspartate 94 in GyrA of *M. tuberculosis* and *M. smegmatis*.

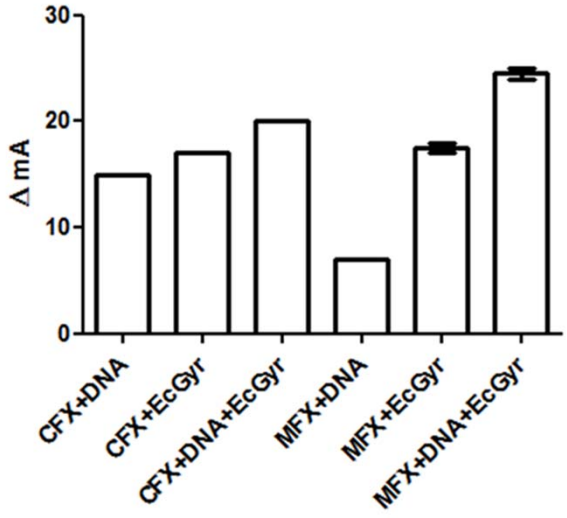

FIG S6. Interaction of FQs with DNA, *E. coli* gyrase (EcGyr) and the EcGyr-DNA complex at lower salt concentration. 20 nM of the FQ was incubated with salmon sperm DNA (200 bp/FQ) or 20 nM of the enzyme or pre-incubated enzyme-DNA complex in the presence of binding buffer containing 25 mM KCl.

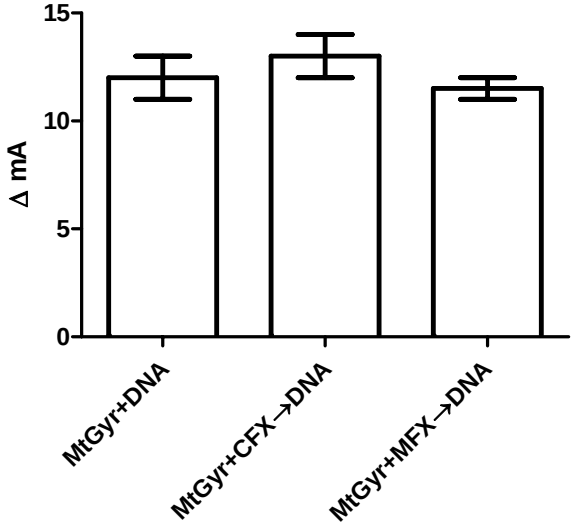

FIG S7. Effect of FQs on gyrase-DNA interaction. 250 nM of *M. tuberculosis* DNA gyrase (MtGyr) was pre incubated with 2.5 μM of CFX or MFX on ice for 10 min. The anisotropy measurements were carried out afte addition of pre-formed enzyme-drug complex or enzyme alone to 10 nM of fluorescein (Ex/Em-492/514 labeled 72 bp DNA.

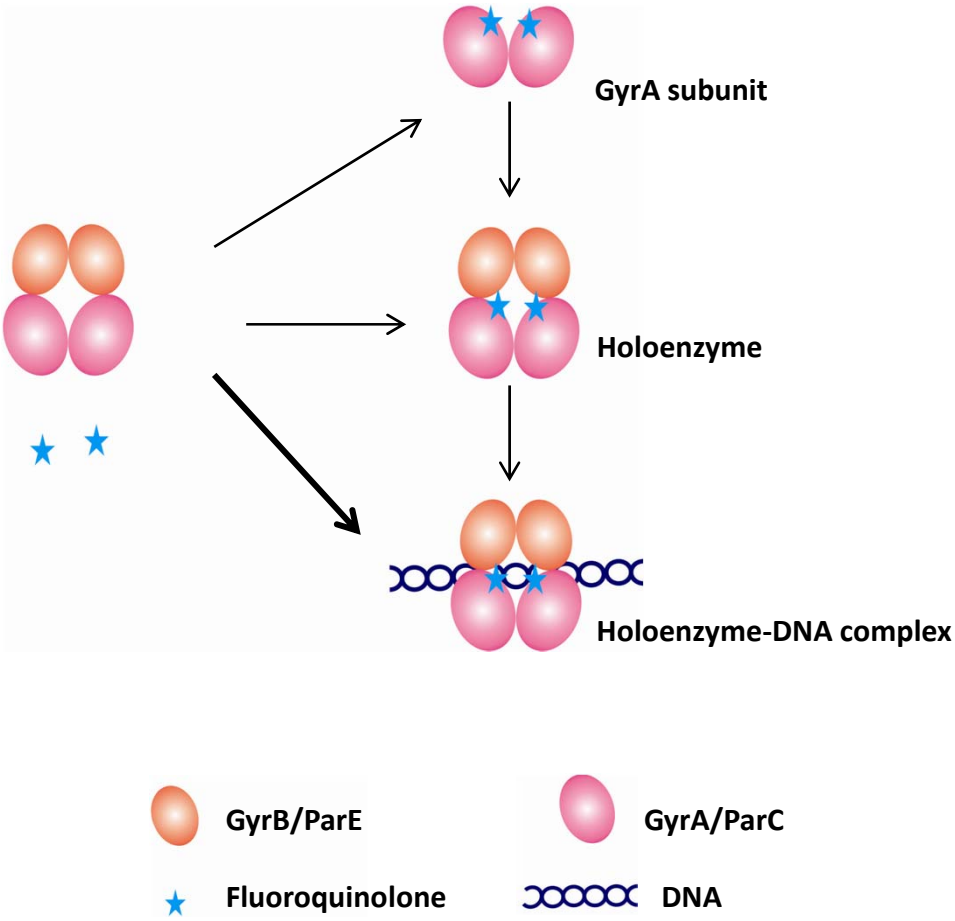

FIG S8. Different modes of FQ binding to the type IIA topoisomerase. The FQs bind to the gyrase/topo IV –DNA complex. Alternatively, the FQs bind to holoenzyme (*E. coli* and mycobacteria) in the absence of DNA. The FQs may bind to the GyrA subunit alone (mycobacteria) that may form a holoenzyme followed by DNA binding. Among the different modes shown, the binding to holoenzyme-DNA complex appears to be the most preferred (Shown by bold arrow).

G-Factor	Value representing the polarization characteristics of the diffraction grating
	G=i90/i0
Anisotropy	Degree of fluorescence polarization
	A=i0-i90XG/i0+2Xi90XG
I0	Fluorescence intensity measured with excitation and emission polarizers at 0 °
I90	Fluorescence intensity measured with excitation and emission polarizers at 90 °

TABLE S1. The terminologies and equations used in the calculation of fluorescence anisotropy.