Supplementary Data

Topoisomerase IV-Quinolone Interactions Are Mediated Through a Water-Metal Ion Bridge

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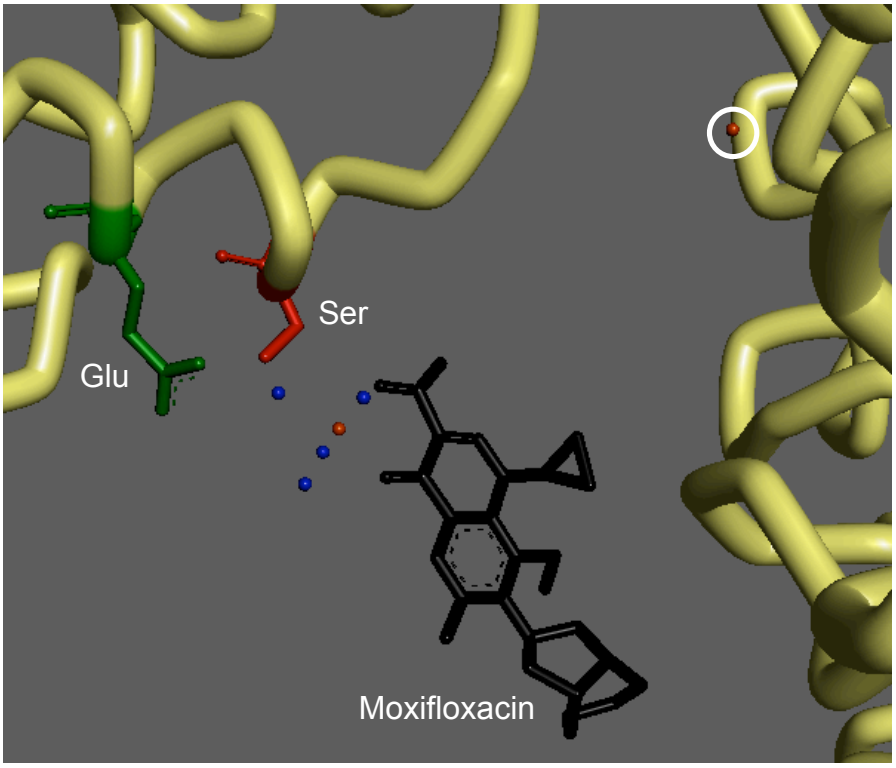
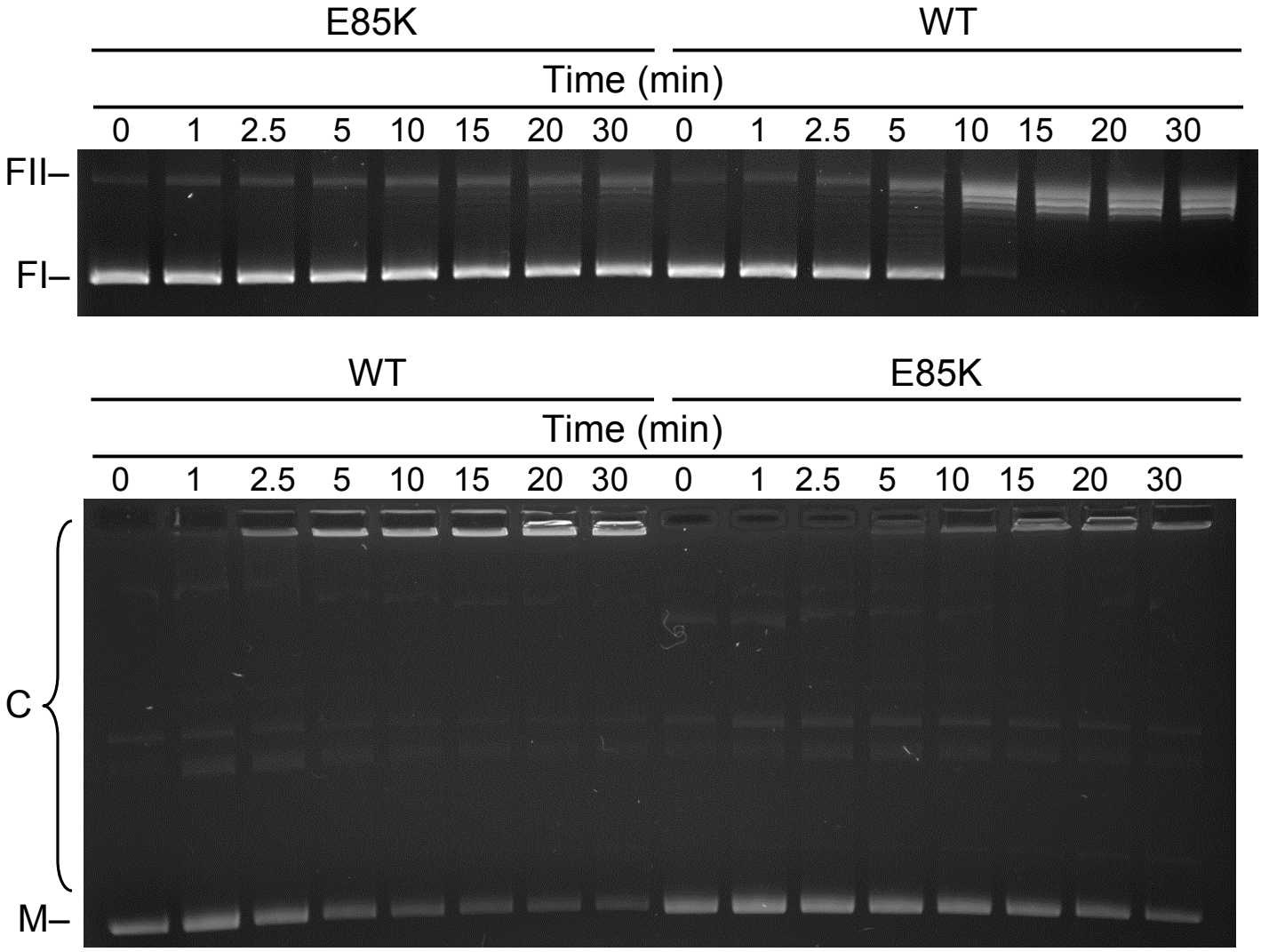
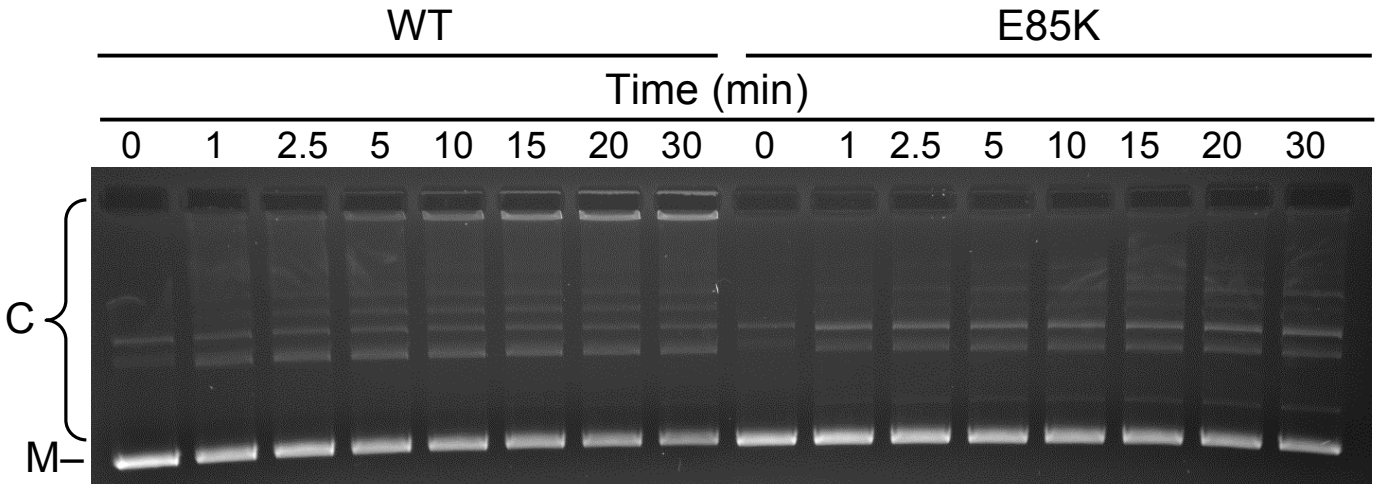


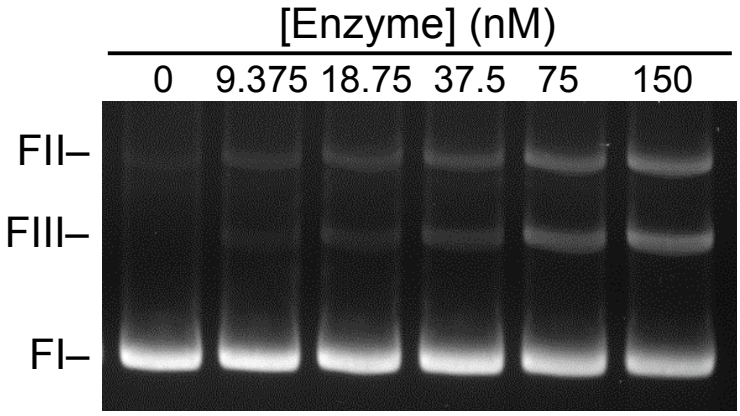
Supplementary Figure S1. Position of moxifloxacin in the *Acinetobacter baumannii* topoisomerase IV cleavage complex crystal structure. Moxifloxacin is shown in black. The catalytic and non-catalytic Mg^{2+} ions are shown in orange, and a white circle denotes the catalytic Mg^{2+} ion occupying metal ion site B. Catalyic metal ion A was not present in this structure. Water molecules filling out the coordination sphere of the non-catalytic Mg^{2+} ion chelated by the C3/C4 keto acid of the quinolone are shown in blue. The backbone of selected portions of the protein amino acid chain is shown in yellow. The side chains of the serine (Ser84) and glutamic acid (Glu88) residues that coordinate with the water molecules in the proposed water-metal ion bridge are shown in red and green, respectively. For clarity, DNA has been omitted from the picture. Protein Data Bank accession 2XKK was visualized using Discovery Studio 3.5 Visualizer (Accelrys Software Inc.). Adapted from Wohlkonig et al. (34).



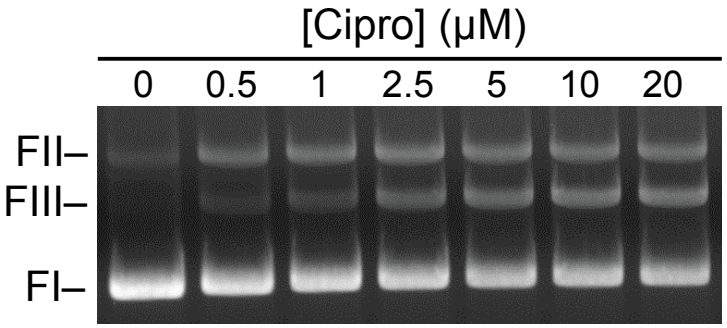
Supplementary Figure S2. Representative gels of DNA relaxation (top) and catenation (bottom) assays carried out in the presence of ATP with GrlA^{E85K} (E85K) and wild-type topoisomerase IV (WT). Relaxation assays were quantified by the loss of the supercoiled (FI band) substrate. FII denotes the position of nicked plasmid DNA. Catenation assays were quantified by the loss of the relaxed monomeric (M) band or by the appearance of catenated (C) products retained in the wells. Both quantification methods yielded similar results.



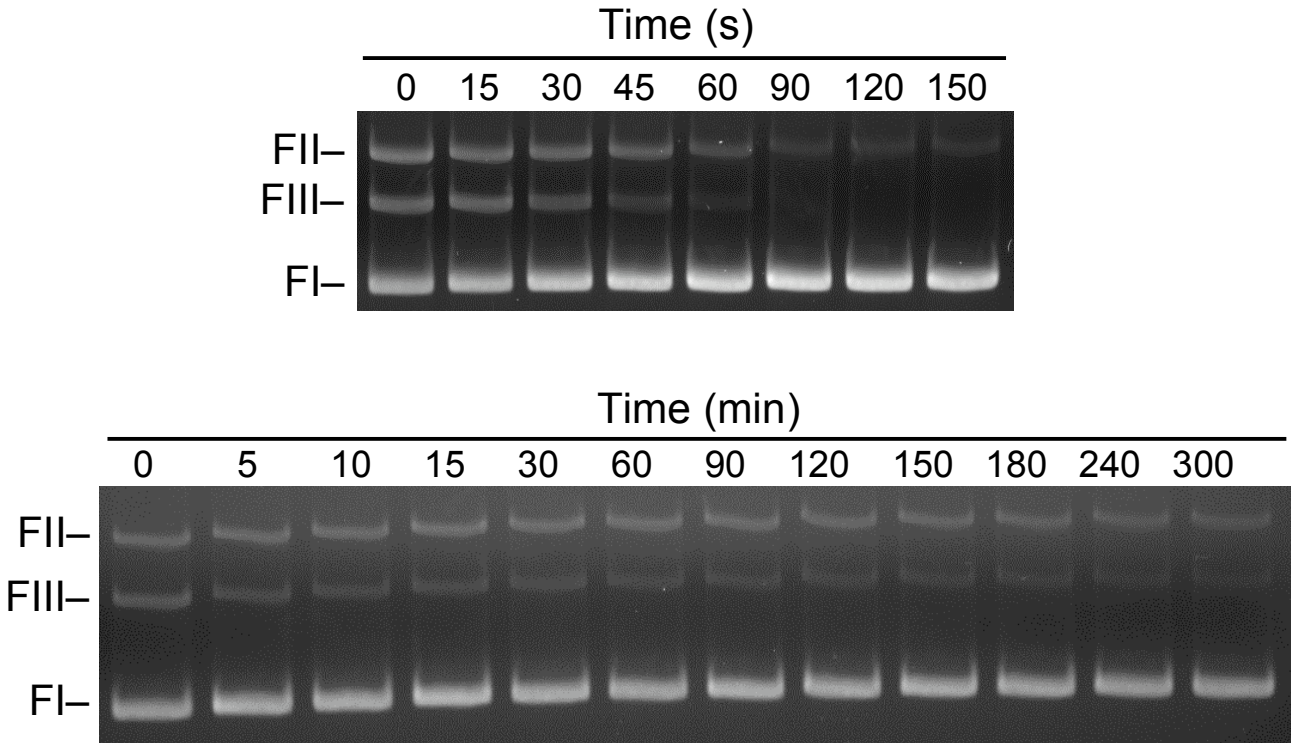
Supplementary Figure S3. Representative gel of DNA catenation assays carried out in the presence of the non-hydrolyzable ATP analog APP(NH)P with wild-type (WT) and GrlA^{E85K} (E85K) topoisomerase IV. Non-turnover catenation assays were quantified by the loss of the relaxed monomeric (M) band or by the appearance of catenated (C) products retained in the wells. Both quantification methods yielded similar results.



Supplementary Figure S4. Representative gel of a DNA cleavage assay carried out in the presence of 10 mM $CaCl_2$ and increasing concentrations of GrlA^{E85K} topoisomerase IV. Cleavage was quantified by the appearance of linearized (FIII band) plasmid. FI is supercoiled plasmid DNA. FII is nicked plasmid DNA.



Supplementary Figure S5. Representative gel of a DNA cleavage assay carried out in the presence of increasing concentrations of ciprofloxacin (Cipro) with wild-type topoisomerase IV. Cleavage was quantified by the appearance of linearized (FIII band) plasmid. FI is supercoiled plasmid DNA. FII is nicked plasmid DNA.



Supplementary Figure S6. Representative gels of DNA religation (top) and cleavage complex persistence (bottom) assays carried out in the presence of 200 μM ciprofloxacin with GrlA^{E85K} topoisomerase IV. Assays were quantified by the disappearance of linearized (FIII band) plasmid. FI is supercoiled plasmid DNA. FII is nicked plasmid DNA.