

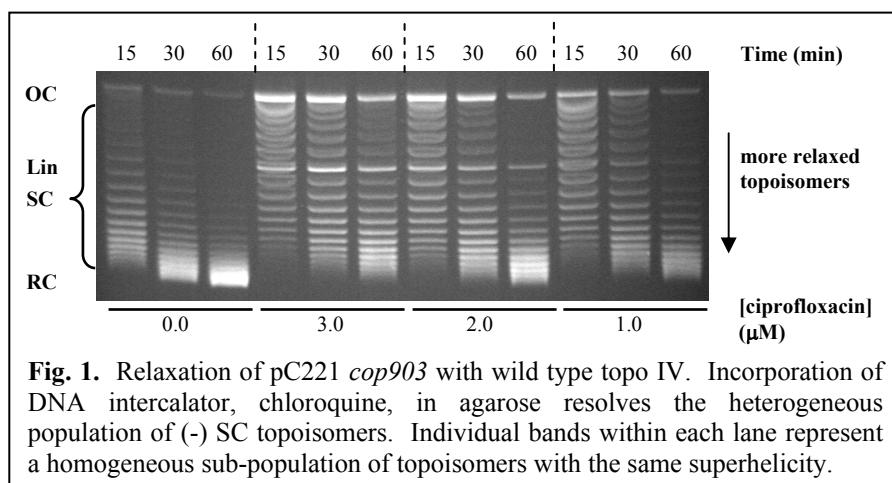
Investigating the interaction of quinolones with topoisomerase IV of *Staphylococcus aureus*

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Background

DNA topoisomerases are ubiquitous enzymes responsible for resolving topological problems arising due to DNA transcription, recombination, replication and chromosome partitioning. Topoisomerase IV (topo IV) of *Staphylococcus aureus* is a heterotetrameric protein composed of two homodimeric subunits: GrlA, which is responsible for DNA binding, the cleavage of both strands (type II class enzyme) and the religation reaction, and GrlB, which binds and hydrolyses ATP allowing enzyme turnover. The action of topo IV results in reduced superhelical density, via the conversion of negatively supercoiled (SC) DNA into the relaxed, covalently closed form (RC).

We have developed an *in vitro* assay that enables us to measure the relaxation of SC DNA substrate (*staphylococcal* plasmid pC221^{cop903}) over time using in-house expressed and purified topo IV. In the context of this assay, we have verified that quinolone drugs such as ciprofloxacin cause significant reduction in the catalytic efficiency of topo IV of *S. aureus* *in vitro*, leading to the accumulation of cleaved DNA complexes, such as nicked open circular (OC) and linear (Lin) forms of DNA (Fig. 1).



Recent Findings

In order to identify the mechanism by which quinolone drugs exert their effect, we set out to identify domains with discrete functions within GrlA. Partial proteolysis of GrlA demonstrated the existence of a stable fragment, which we have cloned, expressed and purified.

Sedimentation velocity analysis demonstrated that the GrlA fragment exists in a monomer-dimer equilibrium. We have also shown that the fragment retains part of the topo IV activity by being able to convert supercoiled and covalently-closed, relaxed DNA substrates to nicked open circular and linear forms independent of GrlB. Crystallisation trials with the fragment have been performed yielding a number of potential targets for X-ray diffraction analysis.

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