

# Crystallographic studies of a type IV topoisomerase from *Staphylococcus aureus*

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## Background

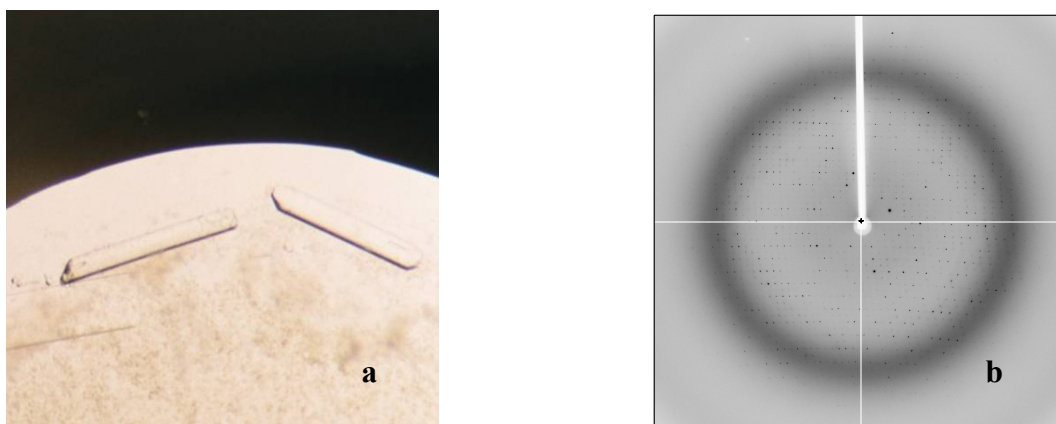
DNA topoisomerases are ubiquitous enzymes responsible for resolving topological problems arising during DNA transcription, recombination, replication and chromosome partitioning. Topoisomerase IV (topo IV) of *Staphylococcus aureus* is a heterotetrameric protein composed of two homodimeric subunits: GrlA, which is responsible for DNA binding, cleavage of both strands (type II class enzyme) and the religation, and GrlB, which hydrolyses ATP allowing enzyme turnover. The action of topo IV results in a reduction in the superhelical density.

Previously, GrlA (90 kDa) had been purified and shown to be functional *in vitro*. With a view to identifying domains with discrete functions within GrlA partial proteolysis was performed and a stable 56 kDa fragment was generated (GrlA56). This was shown to retain DNA nicking activity, but was unable to relegate DNA.

## Recent findings

GrlA56 was subjected to large-scale crystallographic screening using a “Douglas instruments” Oryx 6 robot. Initially small clusters of plate-like crystals were observed, these were readily optimised into large, discrete plates (Fig. 1a).

Diffraction data has been collected at the ESRF (Grenoble, France) to a maximum resolution of 2.8 Å (Fig. 1b). The space group was found to be P2<sub>1</sub> and to contain one dimer per asymmetric unit.



**Figure 1a-** Crystals of GrlA56, **b-** Diffraction data collected from GrlA56 crystals

Molecular replacement using a 59 kDa fragment of a type II topoisomerase from *E. coli* resulted in interpretable electron density, from which 50% of the GrlA56 has been built. Platinyl and selenomethyl derivatives GrlA56 have been produced with a view to generating additional phase information to complete the structure solution.

## Acknowledgements and funding

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