

## INVESTIGATING NONCOVALENT DNA-PROTEIN INTERACTIONS USING ELECTROSPRAY IONISATION MASS SPECTROMETRY

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Chromosome replication in *Escherichia coli* initiates at a specific DNA sequence and is arrested at specific termination sequences (*Ter*). Replication is terminated by interactions between a DNA-binding protein, Tus, and *Ter* DNA sequences. Formation of the Tus-*Ter* complex halts replication forks in one direction but not the other. The X-ray structure of the Tus-*TerB* complex has been solved<sup>1</sup> and revealed interactions between positively charged residues on Tus and the negatively charged phosphodiester backbone of DNA.

Observation of noncovalent complexes using electrospray ionization mass spectrometry (ESI-MS) requires careful consideration of experimental conditions for preparation of the complex (salt concentration, absence of organic solvents), and instrument parameters (source temperature, capillary and cone voltages). The majority of noncovalent complexes examined to date have involved either protein-protein complexes or small ligands bound to proteins. There have been fewer studies of protein-DNA complexes despite the broad significance of protein-DNA binding in biological systems.

We will report here a detailed examination of the noncovalent binding of Tus protein to its terminator sequence, *TerB*, by ESI-MS. A Micromass Q-TOF2 instrument equipped with a Z-spray electrospray ionization source was used for these experiments. The complex was prepared by addition of *TerB*, a double-stranded (ds) 21 mer, to Tus protein (M<sub>r</sub> 35653 Da) which had been dialysed against a range of concentrations of ammonium acetate. Experimental conditions were optimised such that the intact complex could be detected exclusively and with minimal salt and water bound to reduce peak broadening.

Furthermore, spectra obtained for mutant Tus protein-*TerB* complexes and for Tus protein-mutant *TerB* complexes will be presented and compared. These provide evidence of the specificity of binding observed in the gas phase. Finally, the potential of ESI-MS as tool for examining protein-DNA complexes will be discussed.

1. K. Kamada, T. Horiuchi, K. Ohsumi, N. Shimamoto and K. Morikawa (1996) Nature **383**, 598.

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