

Holliday junction resolution is performed by a variety of structure-specific endonucleases as a key step in DNA recombination and repair. It is believed that all resolvases carry out their reaction chemistries in a similar fashion utilizing a divalent cation to facilitate the hydrolysis of the phosphodiester backbone of the DNA, but their architecture varies. To date, with the exception of bacteriophage T4 endonuclease VII, each of the known resolvase enzyme structures has been categorised into one of two families: the integrases and the nucleases. We determined the structure of the *Escherichia coli* RusA Holliday junction resolvase using data collected at the ESRF from crystals of selenomethionine incorporated protein.

The crystals had a primitive monoclinic form and belonged to space group  $P2_1$  with cell dimensions  $a=45.4\text{\AA}$   $b=50.0\text{\AA}$   $c=49.6\text{\AA}$  and  $\beta=101.4$  and there were two copies of the monomer in the asymmetric unit<sup>1</sup>. The selenium substructure was determined from data collected in a full three wavelength MAD experiment on station BM14 at the ESRF using the program SHELXD after data processing through the HKL suite. Phases and maps were generated at  $2.5\text{\AA}$  resolution using the CCP4 suite of programs and model building carried out using TURBO-FRODO. Subsequently, the model was refined using REFMAC5 and the initial data set replaced with a higher resolution set collected to  $1.9\text{\AA}$  on station ID14-4 at the ESRF.

There are two monomers of RusA in the asymmetric unit of the crystal and each is comprised from a 4-stranded mixed  $\beta$ -sheet flanked on one side by 2  $\alpha$ -helices<sup>2</sup>. An obvious dimer is formed by the two monomers through extension of the  $\beta$ -sheet across a non-crystallographic two-fold axis. The structure suggests that dimer formation is essential for binding the  $\text{Mg}^{2+}$  cation utilised in catalysis and that like the other resolvases, RusA distorts its Holliday junction target upon binding. Key residues identified by mutagenesis experiments are well positioned to interact with the DNA.

1. Muranova TA, Sedelnikova SE, Leonard PM, Pasquo A, Bolt EL, Lloyd RG, Rafferty JB. Crystallization of RusA Holliday junction resolvase from *E. coli*. *Acta Cryst D* (2003) 59, 2262-2264.

2. Rafferty JB, Bolt EL, Muranova TA, Sedelnikova SE, Leonard P, Pasquo A, Baker PJ, Rice DW, Sharples GJ, Lloyd RG. The structure of *Escherichia coli* RusA endonuclease reveals a new Holliday junction DNA-binding fold. *Structure* (2003) 10, 1425-1433.