Using data collected at the ESRF, we have determined the structure of the enzyme RecU from Bacillus subtilis that is the general Holliday junction resolving enzyme in Gram-positive bacteria. Data were collected at the ESRF on selenomethionine incorporated and mercury derivatized crystals, the latter being essential for structure determination as the selenomethionine residues turned out to be mostly disordered in the structure. Complete MAD and single wavelength data sets were collected to a resolution of 2.4Å and 3.0Å for the selenomethionine and mercury crystals, respectively. Heavy atom sites were located using SOLVE. The enzyme fold reveals a striking similarity to a class of resolvase enzymes found in archaeal sources and members of the type II restriction endonuclease family to which they are related. The structure confirms the presence of active sites formed around clusters of acidic residues that we have also shown to bind divalent cations. Mutagenesis data supports the key role of certain residues identified from the structure combined with sequence alignments. The RecU structure suggests a basis for Holliday junction selectivity and how sequence specific cleavage might be achieved. Models for a resolvase-DNA complex generated on the basis of the free enzyme structure address how the enzyme might organize junctions into an approximately fourfold symmetric form.
A manuscript reporting on the work and structure is currently “In Press” at Structure.