| ESRF | **Experiment title:** BAG Barcelona – Plasmid conjugative protein NΔ70 TrwB $Ta_6Br_{12}^{2+}$-derivative data collection. | **Experiment number:** LS-1377/78 |
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**Report:**

Genetic evidence shows that TraG-like proteins, the best conserved proteins among plasmid conjugative transfer systems, are involved in the connection between relaxosome and DNA transport complex. They are hence known as "coupling proteins". The available biochemical studies are scarce, presumably because of the inherent difficulties in the purification and analysis of integral membrane proteins. TrwB is the coupling protein of conjugative plasmid R388 and its cytoplasmic domain is expected to interact with the R388 relaxosome. A plasmid was constructed that encodes the soluble domain of protein TrwB (called TrwBΔN70) by deletion of the N-proximal transmembrane segments. TrwBΔN70 could be overexpressed and purified as a soluble protein. The purified protein bound tightly a fluorescent ATP analogue, TNP-ATP, with a $K_S$ = 8.7 μM, in accordance with the ATP-binding signature in its amino acid sequence, but did not show measurable ATPase or GTPase activity. A single ATP binding site was found per TrwB monomer. An intact ATP-binding site was essential for R388 conjugation, since a TrwB mutant with a single amino acid alteration in the ATP-binding signature (K136T) was transfer-deficient. TrwBΔN70 protein was also shown to bind DNA in a non-specific manner. Interestingly, DNA binding resulted in enhanced TrwC *nic*-cleavage activity. This result provides the first evidence that directly links TrwB with conjugative DNA processing. Since DNA bound by TrwBΔN70 also showed increased negative superhelicity (as shown by increased sensitivity to topoisomerase I), it is assumed that *nic*-cleavage enhancement is a result of an increased single-stranded nature of the DNA around *nic*. The mutant protein TrwB[K136T]ΔN70 was indistinguishable from TrwBΔN70 with respect to

these properties, indicating that TrwB ATP-binding activity is not required for the above activities. The reported properties of TrwB suggest potential functions of the TraG protein family in bacterial conjugation, both as a trigger of conjugative DNA processing and as a motor in the transport process.

We have managed to crystallize TrwB lacking the 70 N-terminal residues in three crystal forms, two monoclinic and one trigonal, each with a number of this 48-kDa monomer in the asymmetric unit, ranging from between 6-8 (trigonal form) to 8-10 (big monoclinic cell). Native data are available for the trigonal form (to 2.6 Å resolution) and the big monoclinic cell (to 2.5 Å resolution). Initial phases from a $Ta_6Br_{12}^{2+}$-derivative MAD experiment carried out at DESY for the trigonal crystals rendered some initial phases to 6 Å that, however, have not permitted to localize the local symmetry operators, whose value of the rotation matrix has been established by self rotation calculations.

Using ID02B synchrotron radiation, a 3.5 Å $Ta_6Br_{12}^{2+}$-derivative dataset corresponding to the small monoclinic cell crystal form could be measured and processed. The cell constants are a= 95.1 Å, b= 154.4 Å, c= 103.4 Å, $\beta$= 113.2 °. The $R_{merge}$ of the data is 0.128 and the completeness 91.5 % (whole range) and 84.6 % (last shell, 3.69 – 3.50 Å).

In order to render a further derivative, the protein is being overexpressed as a SeMet-derivative. This, however, will deliver a tricky problem as there are 8 methionines in a monomer and, thus, between 48 and 80 putative SeMet sites (depending on the crystal form) to be localized in the asymmetric units. Two MAD experiments are requested time for, for a $Ta_6Br_{12}^{2+}$-derivative of the big monoclinic cell and for a SeMet-derivative of the trigonal form.