ESRF	**Experiment title:** **BAG Barcelona.**	**Experiment number**: LS-1936
Beamline: ID 14-2	**Date of experiment**: from: 01.05.2001 to: 02.05.2001	**Date of report**: 31-Aug-2001
Shifts: 3	**Local contact(s)**: Ed Mitchell	*Received at ESRF:*

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Report:

Bacterial conjugation is a special case of macromolecular trafficking between cells and comprises plasmid DNA-translocation across membranes through a type IV secretion system. Therefore, a coupling protein is required to bring together the relaxosome with the transport apparatus during cell mating. Such a protein is *E. coli* plasmid R388 TrwB, a basic integral inner-membrane nucleoside-triphosphate-binding protein, capable of non-specific DNA-binding. TrwB is the structural prototype for the type IV secretion system coupling protein family of indispensable proteins, potential triggers of macromolecular transport between cells and export.

Crystals belonging to two space groups, $P3_121$ and $P2_1$, were obtained from a soluble variant of TrwB, TrwBΔN70, lacking its first 70 transmembrane residues and its structure was solved by MAD employing a tantalum bromide derivative [1]. The structure unveils an elongated molecule (Fig. 1a), subdivided into a nucleotide-binding domain reminiscent of RecA protein, other DNA/RNA helicases and AAA ATPases, and a smaller all-α domain [2]. In the trigonal crystal form, six equivalent protein monomers related by non-crystallographic symmetry feature a spherical quaternary structure evocating F_1-ATPase (see Fig. 1b). A central channel traverses the hexamer and connects the cytoplasm with the periplasm. Two such homohexamers are present in the monoclinic crystals. We managed to get structures of the protein in several states, mimicking the apo form, substrate complexes and product complexes [3]. Therefore, diffraction data

of complexes of the protein with the non-hydrolysable ATP and GTP analogues ADPNP and GDPNP, respectively, were collected, so as of complexes with ADP/Mg^{2+} and a sulphate anion. The nucleoside-triphosphate-binding sites are located at the interfaces between protomers. Upon substrate-binding and putative hydrolysis, conformational changes translate this signal from the external surface to the interior central channel, suggesting possible working mechanisms [1,3]. After recruitment of the relaxosome, at least three working mechanisms for TrwB and other T4CPs can be thought of: As a motor pushing the T-DNA to the transport apparatus, as a simple bridging protein between two macromolecular assemblies (the relaxosome and the type IV transport machinery), or as a true DNA pump that threads the T-strand through the pore connecting the donor and the recipient cells.

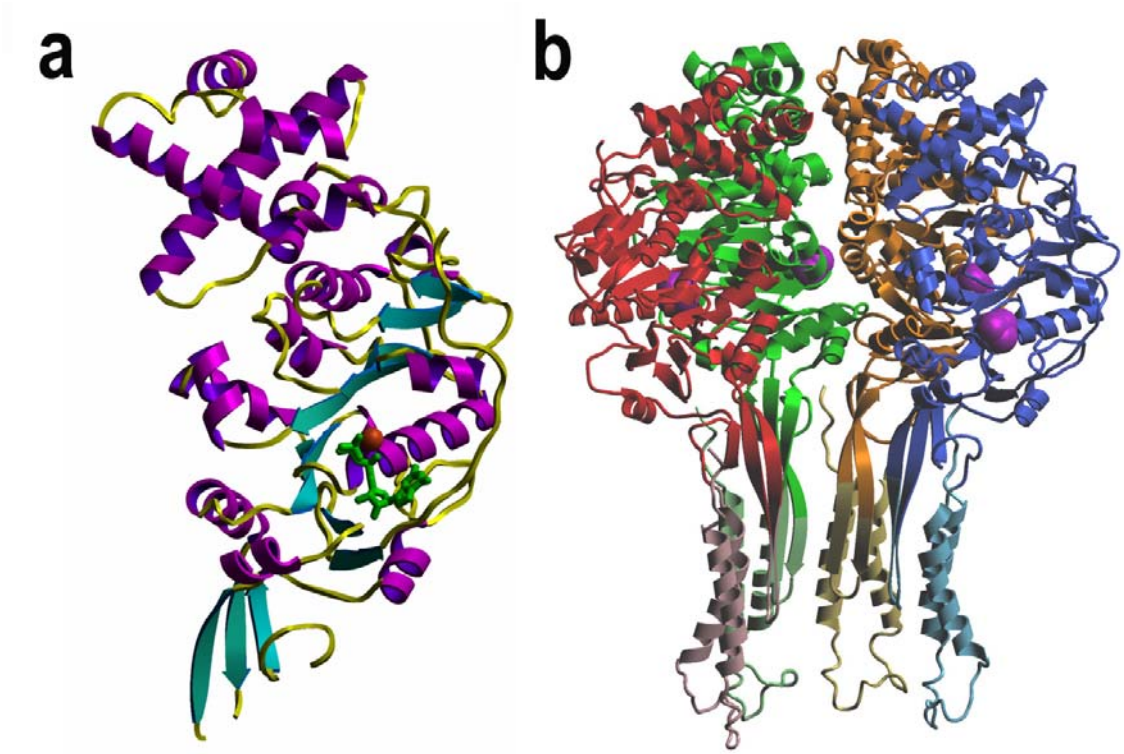

Fig. 1 – (a) Ribbon-plot of a TrwBΔN70 monomer. Helices in magenta, strands as cyan arrows, coils and turns as yellow ropes. A bound GDPNP (substrate complex) molecule is shown as a green stick model to position the NBS. **(b)** Ribbon representation of the complete TrwB, including the modelled transmembrane parts (lighter colours within each chain), showing only four of the six protomers for clarity.

[1] Gomis-Rüth, F.X. and Coll, M. (2001) Solving a 300-kDa multimeric protein by low-resolution MAD phasing and averaging/phase extension. *Acta Crystallogr. sect. D*, **57**, 800-805.

[2] Gomis-Rüth, F.X., Moncalián, G., Pérez-Luque, R., González, A., Cabezón, E., de la Cruz, F. and Coll, M. (2001) The bacterial conjugation protein TrwB resembles ring helicases and F1-ATPase. *Nature*, **409**, 637-641.

[3] Gomis-Rüth, F.X., Moncalián, G., de la Cruz, F. and Coll, M. (2001/2). Mapping the active site cleft of conjugative plasmid protein TrwB, an integral-membrane type IV secretion system coupling protein. Submitted.

ESRF	**Experiment title:** BAG Barcelona - Human Rhinovirus 2-LDL-Receptor complex (2)	**Experiment number**: LS1936
Beamline: ID14-3	**Date of experiment**: from: 1-may-01 to: 2-may-01	**Date of report**: 28-Aug-01
Shifts: 3	**Local contact(s)**: Edward. Mitchell	*Received at ESRF:*

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Report:

Crystals of about 0.1x0.05x0.05 mm in size diffracted to 3 Å resolution, but were stable on the X-ray beam for only 1-2 exposures (see report BAG Barcelona - Human Rhinovirus 2-LDL-Receptor complex (1), date of experiment: 23/09/00-25/09/00) The unit cell, characterized using 0.3º rotation diffraction images was consistent with a $P2_12_12$ space group with parameters: a= 313. b= 348., c= 381 Å.
Partial data set was collected at from 50 crystals, mounted on sealed capillaries. Data collection statistics is shown in table I.
This data was merged with the previous data obtained in ID14.3 (see report BAG Barcelona - Human Rhinovirus 2-LDL-Receptor complex (1)) and used to refine the structure. Refinement is in progress.

Table I
Data collection statistics from the HRV2-VLDLR crystals

• Resolution	3 Å
• Number of crystals	50
• Number of images	50
• Number of reflections	136576
• Number of independent reflections	106574
• R-merge %	19
• Completeness %	16.4