ESRF	**Experiment title:** Bacteriophage phi29 connector Project carried out : $Ta_6Br_{12}^{2+}$-derivative data collection.	**Experiment number:** LS-1065
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Names and affiliations of applicants (* indicates experimentalists):

Alicia Guasch Mitjans, Research Scientist, Institut de Biologia Molecular de Barcelona, CID CSIC, c/ Jordi Girona, 18 – 26, 08034 Barcelona (Spain).*
F. Xavier Gomis-Rüth, Research Scientist.*
Joan Pous Ramos, Ph.D. student.*
Miquel Coll, Group Leader.*

Report:

During the assembly of the head of the double-stranded DNA bacteriophages a region called connector or portal structure plays an important role in the first steps of assembly and the packaging of DNA. This region is also involved in the process of DNA transfer into the host cell during viral infection. This region connects the head of the virus with the tail. Electron microscopy studies, based on two-dimensional projections, carried out by our collaborators at the Centro Nacional de Biotecnología (Madrid), show that the connector has a cylindrical shape with 12 subunits enclosing a 40 Å diameter channel in the center. In the bacteriophage phi29 this large multimeric structure is formed by 12 the subunits of the 35 kD protein p10. Thus the whole connector has a molecular weight of aprox. 420 kD. The protein has been overexpressed and purified.
The connector particle from dsDNA bacteriophage phi29 has been crystallized. The crystals were grown from drops containing an alcohol solution and paraffin oil. Initially, crystals of form I were obtained. They diffracted at least to 3.4 A at beam line BW7B although the diffraction is very weak beyond 7 A. A data set was collected and processed with 60% completeness to 7 Å and 25% complete to 3.7 Å. The space group is C2 and the unit cell dimensions are a=416.86, b=227.62, c=236.68 Å and β=96.3, with four connector particles in the asymmetric unit.

A second type of crystals (crystal form II) have been obtained recently. They belong to space group $P22_12_1$ and have unit cell dimensions of a=170.2, b=170.2 and c=156.6 Å. They display

merohedrol twinning rendering a pseudo $P4_32_12$ symmetry. A preliminary native 3.2 Å data set was collected on BW7B (DESY).
These crystals have been tested on ID02B as well, showing diffraction spots close to 3 Å resolution. However a rather high mosaicity is observed, probably due to the freezing procedure.

Crystal form II is similar to the one analyzed by EM and therefore the orientation of the particles is known. The self-rotation function confirmed the orientation of the 12-fold axes parallel to the crystallographic c axis. Cross-rotation and translation functions have been calculated with a starting model based on the electron microscopy reconstructions. The orientation of the particles from the cross rotation is consistent with the results of the self-rotation function.

Although the orientation of the particles has been determined in both crystal forms, the position of the particles in the unit cell is less certain, since the translation functions calculated using a low resolution EM model did not give a clear solution. Native Patterson maps were also not conclusive. Two aproaches are planed in order to solve the structure. On one hand an improved native data set has to be collected including the low resolution (i.e. 60-20 Å), necesary for molecular replacement techniques with EM models. On the other hand heavy atom derivatives (initially tetrakis and $Ta_6Br_{12}^{2+}$) have been prepared.

$Ta_6Br_{12}^{2+}$ soaked crystals could be measured and a complete dataset to 7 Å resolution could be collected at ID02B. The cell constants are a=171.5, b=171.5 and c=157.8 Å. The dataset display an overall R_{merge} of 0.136 and a completeness of 90.4 % for the whole range (40.0 – 7.0 Å) and of 84.4 % for the last shell (7.25 – 7.00 Å).