ESRF	**Experiment title:** Metal-free hydrogenase from methanogenic archaea	**Experiment number**:
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Shifts: 3	**Local contact(s)**: Dr. Sigrid Stuhrmann	*Received at ESRF:*

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Report:

In some methanogenic archaea an unusual type of hydrogenase (Hmd) has been identified, which catalyzes the reversible hydride transfer from molecular hydrogen to methenyl-H_4MPT. The most interesting feature of this enzyme is the capability to split hydrogen without any redox-active transition metals. This is in contrast to all other hydrogenases known.
The structure of both the enzyme itself and its recently found small molecular weight cofactor is not known yet. Our aim is to determine the crystal structure which should allow a deeper understanding of this unique biochemical reaction.

To achieve this aim, we follow two different strategies. First, we produce the apoprotein from different source organisms as SeMet proteins in *E. coli*. At BM14, Hmd-SeMet crystals from *Methanopyrus kandleri* and *Methanococcus jannaschii* were tested. Due to their limited diffraction power and the rather weak beam at BM14, it was not possible to collect MAD data from these crystals.

The second strategy involves crystals of the native enzyme purified from *Methanobacterium thermoautotrophicum* cells. These were treated with mercury compounds to prepare heavy-atom derivatives suitable for phasing. Upon heavy metal treatment, changes in the space group and the cell parameters prevent application of SIR phasing. Therefore our main interest was collection of SAD or MAD data from these type of crystals.

We collected complete data sets from three different crystals of space group P6 and unit cell dimensions of approx. 138 Å, 138 Å, 99 Å/90°, 90°, and 120° after treatment with mercury at the Hg L_{III} peak wavelength (1.002 Å). After anomalous scaling, data set 1 was 99.8% complete in the resolution range between 50-3.2 Å with R_{merge} of 11.4%; data set 2 was 96.4% complete in the resolution range between 50-3.0 Å with R_{merge} of 8.6%; and data set 3 was 100% complete in the resolution range between 50-2.9 Å with R_{merge} of 9%.

We calculated anomalous Patterson maps from these data, but no significant peaks which could indicate sites with reasonable heavy-atom occupancy could be found. For this reason we did not collect complete MAD data from one of these crystals.