

**Experiment title:**PROTEIN CRYSTALLOGRAPHY AT AFMB-CNRS,  
MARSEILLE**Experiment****number:**

LS1657

**Beamline:**

BM14

**Date of experiment:**

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**Date of report:**

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**Shifts:**

1

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**Report:**

Many glycoside hydrolases are modular proteins. Modules are defined as structural and functional independent subunits of a polypeptide chain. The modules, the most often associated with glycoside hydrolase catalytic domains are the so-called "Carbohydrate Binding Modules" (CBM's). They display high affinities for the oligosaccharidic substrate of the corresponding catalytic domain. The xylanase U (xylU) from *Clostridium thermocellum* consists of four modules, one of which is a CBM (family 6). No structure is available for this type of CBM to date. We have recently crystallized the CBM6 of xylU and have obtained needle-formed crystals with space group P6<sub>1</sub>22 or P6<sub>5</sub>22 and unit cell parameters a=b=60 Å and c=158 Å. A seleno-methionine replaced protein could be produced rapidly. Unfortunately the only methionine, present in the polypeptide chain is at its N-terminal. Since it is an engineered protein, this methionine is not natural and we tempted a MAD experiment on BM14, despite the eventual possibility that it was disordered.

|  | CBM6 (Se-Met) L1<br>(peak) | CBM6 (Se-Met) L2<br>(inflection) | CBM6 (Se-Met) L3<br>(remote) |
|--|----------------------------|----------------------------------|------------------------------|
| Resolution (Å)                         | 24-2.0 (2.11-2.0)          | 24-2.0 (2.11-2.0)                | 24-2.0 (2.11-2.0)            |
| Wavelength (Å)                         | 0.97966                    | 0.97984                          | 0.85507                      |
| Oscillation range                      | 60                         | 60                               | 60                           |
| Unique reflections                     | 11981 (1666)               | 11231 (1241)                     | 10543 (1208)                 |
| Redundancy                             | 6.6 (6.2)                  | 6.0 (5.4)                        | 6.3 (5.5)                    |
| Completeness                           | 99.3 (98.2)                | 98.9 (96.3)                      | 98.7 (95.8)                  |
| $R_{\text{sym}}$                       | 5.0 (20.4)                 | 4.8 (19.2)                       | 5.8 (21.3)                   |
| $\langle I \rangle / \sigma(I)$        | 9.3 (1.8)                  | 9.0 (1.8)                        | 8.8 (1.6)                    |
| No site was found in the Patterson map |                            |                                  |                              |

The seleno-methionine seems to be disordered; despite the clear anomalous signal indicating the presence of the selenium, the patterson map revealed no site.