ESRF	**Experiment title:** Translation initiation	**Experiment number:** LS1679
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Shifts: 6	**Local contact(s):** Dr. Germaine SAINZ	*Received at ESRF:*

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Report:

Our main interest is the study of macromolecular interactions occurring during initiation of translation and involving the initiator tRNA. All cells possess two types of methionine tRNAs, one of which is specifically devoted to initiation of translation. This tRNA is first aminoacylated by methionyl-tRNA synthetase (MetRS). In Eubacteria as well as in mitochondria and chloroplasts, the amino group of methionine esterified onto the initiator tRNA is specifically modified by the addition of a formyl group, catalyzed by methionyl-$tRNA_f^{Met}$ transformylase (formylase). This modification allows tRNA to be recognized by initiation factor 2 (IF2) and positioned at the ribosomal P site for translation initiation. In Eukaryotes as well as in Archaea, this formylation step is absent, and the tRNA is recognized by the initiation factor eIF2, a heterotrimeric protein.

During the first period of this new BAG project, the following datasets have been collected:

1) Methionyl-tRNA synthetase from *E. coli* complexed with methionyl-phosphinate (3 s /1°)
   Cell parameters: 78.5 Å, 45.3 Å, 86.5 Å ß=107.5° ($P2_1$)
   Completeness: 95.4 %
   Multiplicity: 2.5
   Resolution limit: 2.4 Å
   Rsym (outer shell): 3.7 % (10.6%)
2) P12K (C-terminal domain of archaeal MetRS) + $EuCl_2$ (5s /1° and 0.5 sec/1° for low resolution)
   Cell parameters: 38.31 Å, 38.31 Å, 162.61 Å (P321)
   Completeness: 94 %
   Multiplicity: 6.3
   Resolution limit: 2.3 Å
   Rsym (outer shell): 5.6 % (30%)
3) P12K + $HgSO_4$ (5s /1° and 0.5 sec/1° for low resolution)
   Completeness: 95 %
   Multiplicity: 3.5

Resolution limit: 2.2 Å
Rsym (outer shell): 4.4 % (31%)

4) P12K + $K_3UO_2F_5$ (3s /1° and 0.5 sec/1° for low resolution)
Completeness: 98.5 %
Multiplicity: 5.5
Resolution limit: 2.2 Å
Rsym (outer shell): 3.8 % (31%)

5)P12K + PHMB (5s /1° and 0.5 sec/1° for low resolution)
Completeness: 95 %
Multiplicity: 3.9
Resolution limit: 2.2 Å
Rsym (outer shell): 4.3 % (31%)

6) formyl-methionyl-$tRNA_f^{Met}$ (2 x 5s /1°)
Cell parameters: 94 Å, 94 Å, 221.2 Å ($P6_422$)
Completeness: 99.3 %
Multiplicity: 9.7
Resolution limit: 3.3 Å
Rsym (outer shell): 10.5 % (23.2%)

7) eukaryotic-type IF2 from archaea (2 x 5s /1°)
Cell parameters: 128 Å, 128 Å, 225 Å (P321)
Completeness: 99 %
Multiplicity: 5.9
Resolution limit: 6 Å
Rsym (outer shell): 8.3 % (37.8%)

8) Several small crystals were tested. This study was important to orientate future crystallization experiments.

In the field of signal transduction and in that of bacterial resistance to antibiotics we performed the following data measurements:

1- Oxa10 class D β-lactamase. Native at pH 7.5
cell parameters: 66.5 82.3 101.7 90. 95.44 90 ($P2_1$)
completeness: 99.0 %
multiplicity: 3.9
resolution limit: 1.39 Å
Rsym: 0.082

2- Oxa10 class D β-lactamase. Complex n°1 with 6α-hydroxyoctyl penicillanate
cell parameters: 66.5 82.3 101.5 90. 95.39 90. ($P2_1$)
completeness: 95.6 %
multiplicity: 3.6
resolution limit: 1.70 Å
Rsym: 0.107

3- Oxa10 class D β-lactamase. Complex n°1 with 6β-hydroxypropyl penicillanate
cell parameters: 66.1 81.5 106.8 90. 94.48 90. ($P2_1$)
completeness: 99.0 %
multiplicity: 3.7
resolution limit: 1.90 Å
Rsym: 0.052

4- Oxa10 class D β-lactamase. Complex n°2 with 6β-hydroxypropyl penicillanate
cell parameters: 66.6 82.2 101.9 90 95.39 90. ($P2_1$)
completeness: 94.1 %
multiplicity: 3.4
resolution limit: 2.22 Å
Rsym: 0.072

4- Oxa10 class D β-lactamase. Complex n°3 with 6β-hydroxypropyl penicillanate
cell parameters: 65.9 81.4 106.6 90. 94.48 90. ($P2_1$)
completeness: 97.0 %
multiplicity: 3.9
resolution limit: 1.70 Å
Rsym: 0.110
5- Oxa10 class D β-lactamase. Complex n°4 with 6β-hydroxypropyl penicillanate
cell parameters: 66.6 82.5 101.9 90. 95.38 90. ($P2_1$)
completeness: 99.1 %
multiplicity: 3.6
resolution limit: 1.70 Å
Rsym: 0.064
7- Oxa10 class D β-lactamase. Complex n°1 with imipenem
cell parameters: 66.4 82.4 101.8 90. 95.26 90. ($P2_1$)
completeness: 99.9 %
multiplicity: 3.7
resolution limit: 1.80 Å
Rsym: 0.100
8- Oxa10 class D β-lactamase. Complex n°2 with imipenem
cell parameters: 66.3 82.3 101.6 90. 95.37 90. ($P2_1$)
completeness: 97.5 %
multiplicity: 3.6
resolution limit: 1.70 Å
Rsym: 0.066
9- PhoP-N terminal domain. Native.
cell parameters: 45.64, 45.64 135.54, 90 90 90 (P41212)
completeness: 98%
multiplicity: 4
resolution limit: 1.5 Å
Rsym 5%
10- PhoP-N terminal domain. Mercury derivative.
cell parameters: 45.44 45.44 135.2 90 90 90 (P41212)
completeness: 88.6%
multiplicity: 3.8
resolution limit: 2.3 Å
Rsym: 10.8 %
11- TorD. Uranyl derivative
cell parameters: 63.007 92.555 98.299 90.000 90.000 90.000 ($P2_12_12$)
completeness: 86.5%
multiplicity: 4
resolution limit: 4 Å
Rsym: 3.6 %
12- FixJ DNA binding domain
cell parameters: 157.862 157.862 125.606 90.000 90.000 90.000 (P4)
completeness:98%
multiplicity:4
resolution limit:4 Å
Rsym: 15.2 %
13- CpxR DNA binding domain
cell parameters: 40.603 40.014 67.952 90.000 106.148 90.000 ($P2_1$)
completeness: 88%
multiplicity: 3
resolution limit: 2.5 Å

Rsym : 7.2%
14- DivK pH 8.5 native
Cell parameters: 37 Å, 41 Å, 67 Å (P212121)
completeness: 97 %
multiplicity: 7.6
resolution limit: 1.65 Å
Rsym 6.3 %
15- DivK pH 8.5 magnesium bound
Cell parameters: 37 Å, 41 Å, 67 Å (P212121)
completeness: 93 %
multiplicity: 6.5
resolution limit: 1.4 Å
Rsym 6.8 %