ESRF	**Experiment title: Data collection on eavy atom derivatives for the ADP/ATP carrier and on threonine synthase complexes**	**Experime number** LS1924
Beamline: ID14EH1	**Date of experiment**: from: 16 July 2001 to: 17 July 2001	**Date of rep** 21 August 2
Shifts: 1 1/2	**Local contact(s)**: J. MacCarthy	*Received at E*

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Report:

We continued our search for heavy atom derivatives for the ADP/ATP carrier. 35 crystals were tested. Most of them showed a large mosaicity and bad diffraction limits and were not collected. Two derivatives were collected and are still analysed.

Derivatives	Unit cell (Å) and space group	Resolution (Å)	Rsym (%)	Completeness (%)	redundan
Iodine	86.1 86.4 102.7	3.5	10.2	99	3.6

	$P2_12_12_1$				
tungstate	86.2 85.8 100.0 $P2_12_12_1$	3.5	7.2	99	4.8

We had 1.5 shifts in 16 bunch mode. The beamline worked very well during the whole time.

We worked with three types of crystals:

A) Crystals of threonine synthase (TS) soaked with its cofactor pyridoxal phosphate (PLP) and pre-frozen;

B) Crystals of threonine synthase co-crystallised with its reaction product threonine;

C) Crystals of aspartate semialdehyde dehydrogenase (ASDH) for which we want to solve the structure.

A) Crystals soaked in PLP. We tried 4 pre-frozen crystals of which we collected two data sets. All of them were highly mosaic (more than 1 degree), but one data set was integrated and scaled with decent figures. The data were integrated with MOSFLM and scaled with SCALA to 2.7Å resolution.

The table below gives scaling figures:

```
   N 1/d^2 Dmin(A) Rfac  Rfull   Rcum   Av_I   SIGMA I/sigma  sd Mn(I)/sd Nmeas  Nref  Ncent FRCBIAS
Nbias
```

```
  1 0.0137  8.54 0.059  0.091  0.059  13972.  1971.1   7.1   446. 41.9    1360    680     0  -0.308
55
  2 0.0274  6.04 0.056  0.020  0.058   5660.   750.3   7.5   206. 33.9    2538   1269     0  -0.237
107
  3 0.0412  4.93 0.056  0.034  0.057   4820.   640.9   7.5   199. 30.0    3356   1678     0  -0.166
182
  4 0.0549  4.27 0.050  0.052  0.055   6505.   696.5   9.3   265. 30.7    3996   1998     0  -0.074
215
  5 0.0686  3.82 0.055  0.040  0.055   5031.   539.9   9.3   255. 24.6    4612   2306     0   0.004
286
  6 0.0823  3.49 0.068  0.047  0.057   3507.   419.4   8.4   270. 17.3    5210   2605     0  -0.035
366
  7 0.0960  3.23 0.074  0.055  0.059   1967.   253.3   7.8   210. 13.0    5522   2761     0   0.045
406
  8 0.1097  3.02 0.092  0.072  0.060   1184.   175.0   6.8   194.  8.9    6044   3022     0   0.045
433
  9 0.1235  2.85 0.120  0.136  0.062    733.   132.8   5.5   190.  6.1    6394   3197     0   0.032
466
 10 0.1372  2.70 0.172  0.211  0.065    475.   121.2   3.9   192.  4.1    6822   3411     0   0.040
518

 Overall:        0.065  0.059  0.065   3118.   533.3   5.8   225. 16.8   45854  22927     0  -0.067
3034
                  Rfac  Rfull   Rcum   Av_I   SIGMA I/sigma  sd Mn(I)/sd Nmeas  Nref  Ncent FRCBIAS
Nbias


N 1/resol^2   Dmin    Nmeas    Nref   %poss Cm%poss Mlplcty   Rmeas  Rmeas0  (Rsym)    PCV     PCV0
$$
 $$
   1  0.014    8.54     1447     767    97.4    97.4     1.9   0.084   0.084   0.059   0.087   0.087
   2  0.027    6.04     2680    1411    97.6    97.6     1.9   0.079   0.079   0.056   0.082   0.082
   3  0.041    4.93     3514    1836    97.7    97.6     1.9   0.080   0.080   0.056   0.083   0.083
   4  0.055    4.27     4145    2147    97.4    97.6     1.9   0.070   0.070   0.050   0.073   0.073
   5  0.069    3.82     4742    2436    97.5    97.5     1.9   0.078   0.078   0.055   0.081   0.081
   6  0.082    3.49     5322    2717    97.4    97.5     2.0   0.097   0.097   0.068   0.100   0.100
   7  0.096    3.23     5642    2881    97.3    97.5     2.0   0.105   0.105   0.074   0.108   0.108
   8  0.110    3.02     6215    3193    97.1    97.4     1.9   0.129   0.129   0.092   0.135   0.135
   9  0.123    2.85     6529    3332    96.9    97.3     2.0   0.169   0.169   0.120   0.176   0.176
  10  0.137    2.70     6946    3535    96.9    97.3     2.0   0.243   0.243   0.172   0.255   0.255

          Overall     47182   24255    97.3    97.3     1.9   0.092   0.092   0.065   0.095   0.095
                      Nmeas    Nref   %poss Cm%poss Mlplcty   Rmeas  Rmeas0  (Rsym)    PCV     PCV0
```

The map calculated from this data set shows density in the active site that might correspond to the PLP. Refinement is in process.

The second data set gave less good data to only 3.5Å resolution and was not considered any further.

B) Co-crystallisation with threonine. One decent data set was collected. The table below give scaling figures corresponding to the processing with DENZO and scaling with SCALA.

```
N    1/resol^2 Dmin    Nmeas    Nref    %poss Cm%poss Mlplcty  Rmeas  Rmeas0  (Rsym)    PCV
PCV0   $$
 $$
   1  0.014    8.47     1160     725     91.9    91.9     1.6  0.041   0.041   0.029   0.042
0.042
   2  0.028    5.99     2158    1335     92.3    92.2     1.6  0.045   0.045   0.032   0.046
0.046
   3  0.042    4.89     2715    1697     90.9    91.6     1.6  0.051   0.051   0.036   0.052
0.052
   4  0.056    4.24     3231    2029     91.4    91.5     1.6  0.053   0.053   0.038   0.055
0.055
   5  0.070    3.79     3546    2237     90.1    91.1     1.6  0.061   0.061   0.043   0.063
0.063
   6  0.084    3.46     3951    2505     90.3    90.9     1.6  0.078   0.078   0.055   0.081
0.081
   7  0.098    3.20     4230    2687     89.9    90.7     1.6  0.112   0.112   0.080   0.118
0.118
   8  0.111    3.00     4572    2931     89.9    90.6     1.6  0.156   0.156   0.111   0.163
0.163
   9  0.125    2.82     4808    3087     89.3    90.4     1.6  0.232   0.232   0.164   0.245
0.245
  10  0.139    2.68     4011    2659     73.3    87.9     1.5  0.295   0.295   0.208   0.308
0.308
 $$
           Overall     34382   21892     87.9    87.9     1.6  0.075   0.075   0.053   0.078
0.078
                       Nmeas    Nref    %poss Cm%poss Mlplcty  Rmeas  Rmeas0  (Rsym)    PCV
PCV0

    1/d^2 Dmin(A) Rfac  Rfull   Rcum Av_I   SIGMA I/sigma  sd Mn(I)/sd Nmeas  Nref  Ncent
FRCBIAS  Nbias
 $$
  1 0.0139  8.47 0.029  0.020  0.029 11425.   617.5  18.5   401. 34.0     870    435     0  -
0.075     95
  2 0.0279  5.99 0.032  0.028  0.030  5014.   277.5  18.1   199. 28.0    1646    823     0  -
0.016    201
  3 0.0418  4.89 0.036  0.035  0.032  4541.   265.0  17.1   201. 25.1    2036   1018     0
0.003    212
  4 0.0557  4.24 0.038  0.029  0.034  5947.   377.1  15.8   263. 25.7    2404   1202     0  -
0.002    260
  5 0.0697  3.79 0.043  0.036  0.036  4578.   306.9  14.9   254. 20.5    2618   1309     0  -
0.004    281
  6 0.0836  3.46 0.055  0.047  0.039  3253.   278.2  11.7   257. 14.9    2892   1446     0  -
0.030    306
  7 0.0975  3.20 0.080  0.071  0.042  1944.   280.4   6.9   258.  9.2    3086   1543     0  -
0.049    347
  8 0.1114  3.00 0.111  0.107  0.046  1158.   180.5   6.4   254.  6.0    3282   1641     0  -
0.034    349
  9 0.1254  2.82 0.164  0.148  0.050   717.   190.0   3.8   251.  4.1    3442   1721     0  -
0.002    385
 10 0.1393  2.68 0.208  0.306  0.053   567.   158.6   3.6   256.  3.0    2704   1352     0
0.004    315

 Overall:        0.053  0.044  0.053  3080.   279.8  11.0   253. 13.8   24980  12490     0  -
0.020   2751
                  Rfac  Rfull   Rcum  Av_I   SIGMA I/sigma  sd Mn(I)/sd Nmeas  Nref  Ncent
FRCBIAS  Nbias
```

Again, the map calculated from this data set shows density in the active site where the reaction product might be. Refinement is in process to assess if the density remains and can be interpreted by a threonine.

C) ASDH crystals In spite of many trials with different crystallisation conditions, no suitable crystal was found. One data set was collected in order to measure preliminary data. This showed that the crystals are probably monoclinic with unit cell dimensions of around 73 58 103 90. 106. 90.
The integration did not yield useable data.