	Experiment title: **Determination of the 3-D structure of the regulatory subunit of acethydroxyacid synthase isozyme III (RS-AHAS III)**	**Experiment number**: MX-378
Beamline: ID14-4	**Date of experiment**: from: 30/11/04 to: 1/12/04	**Date of report**: 29/12/05
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Boaz Shaanan, *Hagay Shmueli, *Alexander Kaplun**

Interdisciplinary Laboratory for Macromolecular Structure (ILMS)
& Dept. if Life Sciences
Ben-Gurion University of the Negev
Beer-Sheva 84105
Israel

Report:

All data sets were processed using XDS. Data sets from 5 crystals were recorded : 1) MAD data set from Se-Met crystals to 2 Å resolution; 2) Native data set from WT crystal to 1.75 Å; 3) Native data set from an orthorhombic form to 2.5 Å. Crystals 1 & 2 belong to one form of I4 tetragonal crystals whose cell dimensions are described in Table 1. These 3 data sets were used for solving the structure, refining it and for confirming the dimeric structure of the protein (see below). Additionally, two complete SAD data sets to around 2 Å resolution were recorded from crystals of a different I4 tetragonal form (cell dimensions: a,b = 92.8, c= 87.5 Å), however the anomalous signal in those data sets was too low to enable phasing, even though the data looked good. The structure was solved from the MAD data set (#1 above). Of the 8 Se-Met residues in the protein (4 in each chain) 5 were located using SHELX and enabled phasing using SHARP. The chains (the protein is a dimer and crystallizes with a dimer in the a.u. in all forms) were traced nearly to their full length (chain B shows only 158 out of the 163 residues) using the RESOLVE-BUILD script and arp-Warp. For full refinement the native data set to 1.75 Å (#2 above) was used. The current R/R-free are 0.179/0.235. Since the dimeric structure of the protein was somewhat unexpected, it was confirmed using the the data sets from the orthorhombic form (#3 above). This form was solved by molecular replacement and refined to a sufficiently low R-factor. The paper describing the structure in now in press in JMB and will be sent to ESRF as soon as it is published.

During this shift, 3 crystals of valine (the feed-back inhibitor) soaked crystals were mounted, but proved to diffract poorly with clear indications for disorder to the extent that data collection was abandoned.

Table 1. Data collection statistics RS-AHAS III from crystals that were used for structure determination

Statistic	**Se Peak**	**Se Inflection**	**Se Remote**	**Tetragonal WT**	**Orthorombic WT**
Beamline	ESRF ID14-4				
Space group	I4				$P2_12_12_1$
Wavelength (Å)	0.97924	0.97942	0.93928	0.93929	0.93929
Range (Å)	40.0–2.0 (2.10-2.00)			40.0–1.75 (1.80-1.75)	40-2.5 (2.6-2.5)
a (Å)	99.04			98.60	93.6
b (Å)	99.04			98.60	113.1
c (Å)	80.48			80.00	129.5
Total reflections	196,503	197,302	197,793	235,788	234,256
No. unique	51,551	51,607	51,620	38,313	48,189
Completeness %	99.7 (99.1)	99.8 (99.7)	99.8 (99.7)	99.2 (100)	99.9 (100)
I/σ	15.4 (6.6)	14.4 (5.1)	16.0 (5.4)	15.1 (3.3)	16.2 (4.5)
R_{sym} (%)	5.6 (20.8)	6.0 (26.7)	5.4 (25.5)	5.7 (54.1)	6.0 (35.2)
R_{meas} (%)	6.6 (24.2)	7.0 (31.0)	6.7 (39.4)	6.3 (59.0)	6.7 (39.4)