

EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application**:

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.

- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

	Experiment title: Threonine synthase mutant studies	Experiment number: 30-01-402
Beamline: BM30A	Date of experiment: from: 3 february 02 to: 4 february 02	Date of report: 8 March 02 <i>Received at ESRF:</i>
Shifts: 1	Local contact(s): R. Kahn	
Names and affiliations of applicants (* indicates experimentalists): V. Biou * IBS Grenoble C. Mas * IBS Grenoble		

Report:

After solving the structure of apo threonine synthase (TS), we have found soaking conditions that allowed us to see the pyridoxal phosphate cofactor.

We are now working on a mutant whose 36 first residues have been truncated. This mutant has a different behaviour with respect to activator S-adenosyl methionine (SAM) than the wild type enzyme.

The purpose of the present experiment was to soak crystals with SAM or PLP. Some of the crystals had been cocrystallised with SAM.

3 data sets were collected, two of which gave useable electron density maps.

A) crystal soaked in SAM solution for 4 hours

The data is correct to 2.8Å.

Processing with mosflm and scala :

unit cell 76.13 98.31 110.39 90. 90. 90. space group P212121

	Imax	Rfac	Rfull	Av_I	SIGMA	I/Sigma	sd	Nmeas	Nref	Ncent	FRCBIAS	Nbias	Abs.BIAS
	68.	0.672	0.584	30.	31.3	1.0	43.3	58181	9883	1353	-0.039	29921	-1.1
	142.	0.228	0.184	103.	33.8	3.0	43.3	29604	4577	426	-0.011	15829	-1.1
	222.	0.148	0.118	181.	37.4	4.8	43.6	17425	2624	247	-0.022	9351	-3.9
	312.	0.113	0.088	267.	40.9	6.5	44.2	11969	1757	163	-0.027	6478	-7.2
	412.	0.094	0.075	362.	45.9	7.9	45.6	8894	1309	117	-0.026	4780	-9.2
	525.	0.083	0.065	470.	57.1	8.2	47.0	6974	1050	116	-0.025	3757	-11.8
	655.	0.073	0.061	591.	58.1	10.2	49.7	5357	817	82	-0.036	2859	-21.2
	810.	0.068	0.058	733.	65.7	11.2	55.2	4638	701	86	-0.024	2487	-17.5
	999.	0.063	0.057	901.	75.3	12.0	60.2	3584	548	68	-0.026	1906	-23.0
	1243.	0.059	0.051	1117.	86.9	12.9	66.9	3191	490	53	-0.029	1738	-31.9
	1587.	0.054	0.052	1393.	97.6	14.3	78.0	2454	382	43	-0.031	1348	-42.6
	2174.	0.052	0.045	1832.	141.6	12.9	95.2	2080	333	54	-0.025	1088	-47.0
	49250.	0.048	0.046	3077.	204.6	15.0	144.9	1909	345	85	-0.041	942	-124.1
Overall	0.109	0.094	282.	50.9	5.5	47.6	156260	24816	2893	-0.028	82484	-7.9	
	Rfac	Rfull	Av_I	SIGMA	I/Sigma	sd	Nmeas	Nref	Ncent	FRCBIAS	Nbias	Abs.BIAS	
N	1/resol^2	Dmin	Nmeas	Nref	Ncent	%poss	cm%poss	Mlplcty	Rmeas	Rmeas0	(Rsym)	PCV	PCV0
1	0.014	8.31	3772	892	286	98.8	98.8	4.2	0.095	0.095	0.079	0.125	0.125
2	0.029	5.88	9710	1531	301	100.0	99.6	6.3	0.074	0.074	0.068	0.087	0.087
3	0.043	4.80	12909	1937	300	100.0	99.8	6.7	0.076	0.076	0.070	0.092	0.092
4	0.058	4.16	15163	2267	312	100.0	99.9	6.7	0.077	0.077	0.070	0.092	0.092
5	0.072	3.72	17336	2556	305	100.0	99.9	6.8	0.091	0.091	0.084	0.111	0.111
6	0.087	3.39	19097	2803	304	100.0	99.9	6.8	0.120	0.120	0.111	0.147	0.147
7	0.101	3.14	20507	3026	304	100.0	99.9	6.8	0.172	0.172	0.159	0.211	0.211
8	0.116	2.94	21737	3256	302	100.0	99.9	6.7	0.246	0.246	0.226	0.306	0.306
9	0.130	2.77	22424	3449	305	100.0	100.0	6.5	0.373	0.373	0.343	0.516	0.516
10	0.145	2.63	13842	3336	269	92.1	98.8	4.1	0.497	0.497	0.433	0.681	0.681
Overall			156497	25053	2988	98.8	98.8	6.2	0.119	0.119	0.109	0.151	0.151
			Nmeas	Nref	Ncent	%poss	cm%poss	Mlplcty	Rmeas	Rmeas0	(Rsym)	PCV	PCV0

B) crystal cocrystallised with SAM.

Unit cell 75.7, 98.4, 109.4, 90. 90. 90. Space group p212121.

The crystal was less mosaic than the first one and the treatment with mosflm and scala gave an R-merge of 5.7% to 2.6Å with 19.7% in the highest resolution bin; completeness was 98.6%.

The electron density map calculated from this last data set showed extra density in the active site. Model building is in progress.

