

	<b>Experiment title:</b> Metal-free hydrogenase	<b>Experiment number:</b> LS2054
<b>Beamline:</b> ID14-4	<b>Date of experiment:</b> from: 4.5.2002 8.00 to: 5.5.2000 8.00	<b>Date of report:</b> 21.2.2002
<b>Shifts:</b> 3	<b>Local contact(s):</b> Raimond Ravelli	<i>Received at ESRF:</i>
<b>Names and affiliations of applicants (* indicates experimentalists):</b>  Seigo Shima Rudolf K. Thauer Björn Mamat* Ulrich Ermler*		

Report:

### 1. Metal-free hydrogenase

Our aim was to find a crystal which diffracts better than 2.8 Å. Unfortunately, no suitable crystal was found despite of an intensive screening of about 30 promising crystals.

### 2. F<sub>420</sub> dependent alcohol dehydrogenase

The crystals diffracted to around 2.0 Å. However, the collection of a native data set was not feasible, because the radiation damage was too severe. After about 50 frames the diffraction decreases to around 3.0 Å. The strong ID14-4 beamline is not suitable for these crystals at the current stage.

### 3. Dissimilatory sulfite reductase

A native data set to around 2.3 Å resolution could be measured.

Shell	I/Sigma in resolution shells:										
Lower limit	Upper limit	% of of reflections with I / Sigma less than									
		0	1	2	3	5	10	20	>20	total	
20.00	5.39	0.4	0.5	0.8	1.1	1.8	6.6	65.5	33.9	99.4	
5.39	4.30	0.4	0.7	1.3	1.8	3.0	6.4	23.1	76.6	99.8	
4.30	3.76	1.2	2.0	3.1	4.0	6.1	12.1	31.1	68.8	99.9	
3.76	3.42	1.4	2.7	4.4	6.4	10.4	19.9	44.1	55.6	99.7	
3.42	3.17	2.5	5.5	8.9	12.3	18.3	31.9	58.9	41.0	99.8	
3.17	2.99	3.9	7.7	12.5	17.1	25.1	42.3	68.0	31.8	99.8	
2.99	2.84	5.1	11.2	17.7	23.6	33.3	51.9	76.1	23.7	99.8	
2.84	2.71	5.9	13.4	21.7	29.4	41.8	61.4	83.9	16.0	99.9	
2.71	2.61	8.4	18.1	27.8	36.3	49.1	67.5	87.9	11.9	99.8	
2.61	2.52	9.5	21.2	32.8	42.1	54.9	73.7	91.5	8.3	99.8	
2.52	2.44	10.2	24.8	37.9	48.0	61.2	79.7	93.9	6.0	99.9	
2.44	2.37	13.3	30.0	43.9	53.3	65.9	82.2	95.6	4.2	99.8	
2.37	2.31	16.2	33.9	48.0	57.6	69.5	84.5	96.6	3.2	99.7	
2.31	2.25	12.1	27.4	43.1	55.4	70.1	85.7	96.3	3.2	99.5	
2.25	2.20	15.6	35.4	51.5	62.4	74.5	88.5	97.0	1.7	98.7	
2.20	2.15	18.8	42.3	58.5	67.5	77.8	88.9	96.2	1.1	97.3	
2.15	2.11	16.9	39.6	55.0	63.6	73.0	82.8	86.9	0.7	87.7	
2.11	2.07	14.7	35.5	49.8	57.9	66.4	74.9	78.1	0.3	78.4	
2.07	2.03	15.0	35.7	48.5	54.3	60.9	67.0	68.8	0.2	69.0	
2.03	2.00	13.8	32.1	43.2	48.2	52.8	57.0	58.3	0.1	58.4	
All hkl		9.3	21.0	30.5	37.1	45.8	58.2	74.9	19.4	94.3	

Shell	Lower limit	Upper limit	Average I	Average error	Average stat. Chi**2	Norm. R-fac	Linear R-fac	Square R-fac
20.00	5.39	3309.3	182.5	136.7	1.553	0.049	0.068	
5.39	4.30	3025.9	106.7	106.7	2.074	0.050	0.066	
4.30	3.76	2464.6	86.4	86.4	2.007	0.054	0.078	
3.76	3.42	1553.6	61.6	61.6	1.846	0.070	0.373	
3.42	3.17	967.8	46.2	46.2	1.517	0.071	0.072	
3.17	2.99	692.5	39.9	39.9	1.387	0.083	0.080	
2.99	2.84	518.5	36.8	36.8	1.264	0.098	0.290	
2.84	2.71	380.1	35.5	35.5	1.346	0.144	0.238	
2.71	2.61	309.3	35.5	35.5	1.150	0.165	0.872	
2.61	2.52	253.8	36.0	36.0	0.959	0.171	0.145	
2.52	2.44	218.9	37.3	37.3	0.878	0.199	0.163	
2.44	2.37	194.9	38.9	38.9	0.922	0.234	0.232	
2.37	2.31	179.4	41.2	41.2	1.059	0.282	0.424	
2.31	2.25	194.6	44.9	44.9	2.894	0.298	0.000	
2.25	2.20	158.6	46.6	46.6	0.888	0.301	0.385	
2.20	2.15	132.6	48.4	48.4	0.735	0.334	0.272	
2.15	2.11	115.7	51.3	51.3	0.728	0.352	0.286	
2.11	2.07	119.8	53.9	53.9	2.662	0.417	0.000	
2.07	2.03	95.4	55.5	55.5	1.163	0.409	0.794	
2.03	2.00	83.2	57.5	57.5	0.883	0.414	0.483	
All reflections		785.7	57.2	54.8	1.410	0.085	0.143	

#### 4. 4-OH-Benzoyl-CoA reductase

A native data set to 1.7 Å resolution could be collected.

Shell I/Sigma in resolution shells:

Lower limit	Upper limit	% of reflections with I / Sigma less than									total
		0	1	2	3	5	10	20	>20		
20.00	4.60	0.1	0.2	0.4	0.6	1.5	9.8	80.1	5.6	85.7	
4.60	3.66	0.1	0.3	0.5	0.7	1.2	3.5	32.8	57.2	90.0	
3.66	3.20	0.2	0.4	0.9	1.5	2.6	6.6	31.8	59.5	91.3	
3.20	2.90	0.6	1.4	2.4	3.4	5.5	11.9	39.1	53.1	92.2	
2.90	2.70	1.3	2.6	4.1	5.7	9.1	17.9	45.9	47.1	93.0	
2.70	2.54	1.7	3.3	5.4	7.5	11.9	23.0	51.0	42.8	93.8	
2.54	2.41	2.2	4.6	7.2	10.0	15.1	28.0	55.5	38.9	94.4	
2.41	2.31	2.5	5.1	8.3	11.7	17.8	32.2	59.0	36.0	95.0	
2.31	2.22	3.0	6.5	10.4	14.4	21.8	37.6	64.4	31.0	95.4	
2.22	2.14	2.7	6.7	11.7	16.7	25.4	42.8	69.0	26.7	95.6	
2.14	2.07	2.1	6.4	12.3	18.1	28.0	47.0	72.9	23.2	96.1	
2.07	2.02	2.1	6.6	13.2	20.0	31.3	51.3	76.8	19.6	96.4	
2.02	1.96	3.2	9.3	17.0	24.3	36.6	57.6	80.7	15.8	96.5	
1.96	1.91	6.8	16.3	25.9	33.8	46.4	65.4	85.2	11.7	96.9	
1.91	1.87	10.1	21.0	31.5	39.8	52.1	70.4	88.1	8.8	96.9	
1.87	1.83	12.1	24.6	35.9	44.7	57.0	74.8	90.2	6.9	97.2	
1.83	1.79	12.7	27.1	39.2	48.5	61.2	77.8	91.8	5.3	97.1	
1.79	1.76	13.0	29.0	42.1	51.8	64.8	81.1	93.1	4.0	97.1	
1.76	1.73	12.6	29.3	44.0	54.2	67.2	82.2	92.1	2.6	94.7	
1.73	1.70	12.8	30.2	45.6	55.8	67.5	79.6	87.3	1.7	88.9	
All hkl		5.1	11.6	17.9	23.2	31.2	45.0	69.3	24.9	94.2	

Shell limit	Lower Angstrom	Average I	Average error	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac
20.00	4.60	47523.8	3794.1	3288.1	1.703	0.063	0.081
4.60	3.66	58711.4	2875.5	2875.5	2.644	0.056	0.071
3.66	3.20	36214.8	1574.1	1574.1	2.898	0.055	0.066
3.20	2.90	21623.5	973.2	973.2	2.993	0.059	0.070
2.90	2.70	14624.8	703.8	703.8	2.975	0.063	0.072
2.70	2.54	11713.7	615.7	615.7	2.916	0.070	0.153
2.54	2.41	9667.5	551.7	551.7	2.815	0.074	0.080
2.41	2.31	8789.3	556.8	556.8	2.695	0.080	0.087
2.31	2.22	7777.3	562.6	562.6	2.462	0.089	0.222
2.22	2.14	6810.8	575.2	575.2	2.251	0.095	0.103
2.14	2.07	6276.6	602.0	602.0	2.146	0.106	0.116
2.07	2.02	5584.7	620.8	620.8	2.051	0.120	0.138
2.02	1.96	4658.7	624.8	624.8	1.841	0.134	0.150
1.96	1.91	3533.5	625.4	625.4	1.486	0.161	0.213
1.91	1.87	2759.2	618.9	618.9	1.362	0.196	0.206
1.87	1.83	2340.0	621.6	621.6	1.248	0.229	0.231
1.83	1.79	1983.4	633.1	633.1	1.149	0.269	0.283
1.79	1.76	1755.6	646.4	646.4	1.078	0.303	0.319
1.76	1.73	1557.8	667.9	667.9	1.002	0.333	0.372
1.73	1.70	1371.8	702.4	702.4	1.031	0.354	0.424
All reflections		12375.7	935.8	912.8	2.064	0.076	0.081

Another crystal was used to measure a data set at 0.93 Å and 1.28 Å, to obtain anomalous dispersion informations. A measurement at the iron edge is not possible at the ID14-4 beamline.

A third crystal soaked with the substrate 4-OH-benzoyl-CoA was measured. The  $R_{sym}$  value was 7.0 % in the resolution range 2.5 –20.0 Å. The completeness was 97 %.