ESRF	**Experiment title:** Crystal structure analysis of Rnalp by multiple-wavelength anomalous dispersion (MAD) and by multiple isomorphous replacement (MIR)	**Experiment number:** LS - 780 / LS - 781
Beamline: BM 14	**Date of experiment:** from: **2.** July to: 5. July 1997	**Date of report:** **25.** February 98
Shifts : 5 and 4	**Local contact(s):** Dr. Andrew Thompson	*Received at ESRF:* 05 MAR. 1998

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Report:

We were allocated block beam time for MAD and MIR data collection on Rnalp crystals. As crystals are too small to identify derivatives at our local anode, and as we could not grow crystals of the Se-Methionine mutant large enough for x-ray analysis in time, we planned to use the beam time to collect data sets of promising compounds and to start with a complete MAD data collection when identifying a successful candidate.

Rnalp crystals are hemihedrally twinned with twin fractions ranging from 0.0 to 0.5 in different crystals (0.5 means perfect hemihedry). This kind of twinning does not effect R_{symm} and is not visible in the diffraction pattern. When collecting data, the amount of twinning can be estimated by analyzing the intensity distribution statistics. As our best native data set had turned out to be considerably twinned, we decided to collect a new less twinned native data set as a prerequisite for a successful MIR analysis. We succeeded in finding a crystal with a small twinning fraction and collected a complete data set with good intensity statistics up to 2.65 A (see table 1).

table 1: intensity statistics of data sets collected at BM 14

	native	$HgCl_2$	MeHgBr	$KAu(CN)_2$	Me_3PbAc
max. resolution	2.65 A	3.23 A	3.23 A	3.20 A	3.20 A
reflections	149534	78380	68250	56905	40072
unique refl.	24708	13970	13729	14337	13758
completeness (%)	97.8	99.3	99.4	99.6	94.1
I / sigma	18.2 (4.1)	16.2 (7.9)	12.4 (4.3)	19.3 (6.6)	6.2 (2.1)
R symm (%)	8.4 (19.6)	9.3 (20.9)	10.3 (29.1)	6.1 (15.5)	13.3 (31.2)
twin fraction	0.06	0.12	0.23	0.21	0.36

(Values in brackets refer to outer shell)

A detailed twin analyses according to T.O.Yeates [Detecting and Overcoming Crystal Twinning, Methods in Enzymology 276, 344 - 358] resulted in a twin fraction of 0.06 for this new native data set, the lowest twin fraction of all the data sets collected so far. Additionally, we then collected data sets of 4 potential derivatives. They show higher degrees of twinning (see table 1). We could not identify heavy atom sites with these data sets, which is probably due to the fact, that different twin fractions between native and derivative data sets conceal the small differences caused by an incorporated heavy atom. Attempts at finding heavy atom positions after detwinning the data failed so far, possibly because the derivative data sets are too severely twinned.

The problem of non-isomorphism in terms of different twin fractions between native and derivative data could be circumvented by collecting all data on one crystal in a MAD experiment. Therefore we have put all our efforts in optimizing the Se-Met-Rnalp crystals and have succeeded in growing crystals that are large enough for x-ray analysis. This allows us now to determine the twin fraction of a crystal from low resolution data collected at our local anode. Together with the high resolution native data set collected at BM 14, it should now be possible to solve the structure of Rnalp with one MAD experiment in the near future.