| ESRF | **Experiment title:** Protein crystal examination: Histone Hl containing nucleosomes and transcription factors | **Experiment number:** *IS-677* |
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| **Beamline:** | **Date of experiment:** from: 16/10/97 15:OO to: 19/10/97 7:00 | **Date of report:** 19 December, 1997 |
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## REPORT (confidential):

**Histone H1 containing nucleosomes**

Previously in my laboratory, 21 different lengths of a hybrid DNA sequence had been assembled into nucleosomes one at a time, a major fragment of histone H1 added to each, and crystallization attempted. Two different DNA lengths yielded mountable crystals, and these were examined during the LS-677 beam time. 25 crystals were examined in 1 to 3 orientations with exposures times of from l/2 to 3 min. Crystal sizes varied between 30-80 microns in one or two dimensions with a the third dimension of over 100 microns. The wavelength used on ID2 was 0.988 Å and the detector to crystal distance was generally 900 mm. The primary differences between crystals containing one of the two DNA lengths examined were the methods used to introduce cryoprotectant and the subsequent cryocooling procedures. The most significant observations follow. 1) The crystals prepared using sequence 1 did not diffract beyond 13 Å spacings. 2) Several of the crystals incorporating sequence 2 that were cryocooled in Zurich diffracted to 6 Å resolution. These crystals always showed heterogeneous diffraction patterns, however, and were not useful for data collection. 3) Smaller crystals containing sequence 2, which were transported to ID2 in their crystal growth drops and subsequently cryocooled by a different protocol than used in Zurich, diffracted to 7-8 Å spacings. These crystals showed a clean lattice. I expect that the largest crystals in conjunction with the best cryocooling protocol will yield a full data set to 5 or 6 Å resolution. Our interest is to use these crystals to determine the location and orientation of histone H1 in a complete nucleosome.

**Prospects:** Collect a native data set and possibly two heavy atom derivatives sets as soon as possible. We will continue with our attempts to improve the diffraction resolution.

**MEF2A/DNA complex**

MEF2A is a gene activation factor that collaborates with two helix-loop-helix proteins to differentially regulate heart muscle development. Our first crystals in this system contain the MADS and MEF domains of MEF2A bound to a cognate DNA sequence. We used crystals that were no greater than 50x100 microns in the smallest dimensions. Cryocooled crystals diffract to 2.7 Å on our rotating anode generator and to 1.8 Å on ID2.11 crystals were examined, and all but the last gave heterogeneous diffraction patterns. The final crystal was used to collect data to 1.8 Å with an Rmerge of 7% overall and 15% at 1.8-2.0 Å. Unfortunately, this native data set is only 85% complete because the beam time ended before finishing it.

**Prospects:** Collect a native data set and two Iodo (for MIR) and two Bromo (for MAD) derivative sets as soon as possible.

**TBP/TFIIA/TFIIB/DNA**

The TBP/TFIIA/TFIIB/DNA complex is central to the initiation of transcription from TATA-box containing genes (provides current paradigm for gene transcription initiation). 11 crystals from 5-100 microns in their smallest dimensions were exposed to X-rays. The best of these diffracted to 4.2 Å

**Prospects:** Attempt to improve the diffraction from these crystals. Screen more crystals, micro or larger, with the high brilliance beam. ID2 enables us to observe the most useful crystallization conditions to pursue based on microcrystals. This is a very important advance.

**TFIIF**

We have crystallized the heterodimer TFIIF which is an essential part of the basal level transcription complex of eukaryotic cells (new crystals, not covered in our original proposal). We examined 6 different micro crystals on the ID2 and found the best diffracts to 3.5 Å resolution.

**Prospects:** This project is on the verge of requiring full data collection of the native and derivative crystals. We are attempting to improve the crystals, but will, I suspect, require further time using a highly brilliant X-ray beam to characterize them and/or collect data. There are already new crystals available that require examination.

**Conclusions:** Two projects, Hl containing nucleosomes and MEF2A/DNA, are ready for full data collection. TFIIF could go ahead with data collection, but we are still attempting to improve the crystals. The projects TBP/TFIIA/TFIIB/DNA, TFIIF, and nucleosomes all require further beam time for screening crystals on a beamline comparable to ID2.

**Final note:** Nucleosome core particle crystals containing regulatory DNA sequences (nucA and nucB) from the mouse mammary tumor virus (MMTV) 3' promoter (LTR) were observed to diffract isotropically out to 2.5-3.5 Å spacings. These crystals will be the subject of a new proposal.