ESRF	**Experiment title:** Heavy Atom Derivative Data Collections on Signal Recognition Particle SRPΦ14-9 Fusion Protien.	**Experiment number:** Ls261
Beamline: BM2	**Date of experiment:** from: December 06/96 to: December 06/96	**Date of report:** January 10/96
Shifts: 2	**Local contact(s):** Michel Roth/ Pierre LeGrand	***Received at ESRF:***

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Crystal Structure of Signal Recognition Particle SRPΦ 14-9 Fusion Protein

The mammalian Signal Recognition Particle (SRP) is a cytoplasmic ribonucleoprotein particle (RNP) that plays an essential role in the targeting of secretory and membrane proteins to the rough endoplasmic reticulum (RER). Targeting occurs co-translationally and translocation across the RER membrane begins before polypeptide synthesis is complete.

Whereas many functional and biological aspects of SRP are understood, detailed structural information is completely lacking. The crystal structure of SRPΦ14-9 will provide information about protein/protein and protein/RNA interactions as well as being the first step to understand the structural basis of the SRP *Alu* domain mediated elongation arrest.

Objectives:

The objective of this experiment was to collect data at cryogenic temperatures on various heavy atom soaked SRPΦ14-9 crystals for the use in phase determination by multiple isomorphous replacement methods (MIR).

Experimental Outline:

Previous native data collections at the ESRF-BM14, produced good quality data, gave insight into optimum cryo conditions and provided valuable resolution limit information that was used to plan both derivative screening experiments and high resolution native data collection experiments. Crystals diffracting to beyond 2.5 Å resolution have been observed, even though crystals were quite small (50 x 50x 80 $Å^3$).

On BM2 data was collected at cryogenic temperatures (100 K) from a liquid nitrogen cold-strearn due to the small size of SRPΦ14-9 crystals and extreme sensitivity to X-ray radiation aiding in higher diffraction resolution limits and prolonged exposure time in the beam. A monochromatic beam was set to a wavelength of 0.9790 Å to optimize data collections at, or near, the absorption edges of the heavy atoms screened for in this experiment. Various heavy atom derivative solution soaks including platinum, mercury, gold, lead, uranyl, osmium, ytterbium, iridium silver, indium palladium and tungsten were chosen as potential data collection candidates.

Experimental Results:

The data collected at BM2 provided insight into further planning of experiments. Data were collected on three potential derivatives (osmium platinum and uranyl) showing diffracting to beyond 2.6 Å resolution.

Due to very small crystal size yielding low intensity diffraction spots and high background from a large beam (300 μm^2 collimator) combined with prolonged exposures, the data were difficult to process. A single data set (osmium) was integrated with XDS and treated with the CCP4 Program Package. The data was collected to 2.65 Å, but only useable to 3.0 Å. This data yielded R_{sym}s of 8.1'% and multiplicity of 6.0. An anamolous signal was detected in the data indicating that osmium was present and bound in the crystal. The data is only isomorphous to 6.0 Å and low resolution phase calculations are presently underway.

Conclusion:

The experiment provided information on optimum reproducible freezing conditions; a direction to pursue with different derivative solution concentration soaks; the importance of small beam size and of large crystal size.

Presently, we have ideal cryoprotectants and freezing methods, significantly larger crystals (150 x 150 x 300 $Å^3$) which diffract to beyond 2.1 Å, and several potential heavy atom derivatives which bind the protein, as well as selenomethionyl-SRPΦ 14-9 crystals.