ESRF	**Experiment title:** Crystal structure of bovine rhodopsin	**Experiment number:** LS-1193
Beamline: ID13	**Date of experiment:** from: 27-Nov-98 to: 01-Dec-98	**Date of report:** 10-Feb-99
Shifts: 9	**Local contact(s):** Christian Riekel, Manfred Burghammer	*Received at ESRF:* 15 FEV. 1999

Names and affiliations of applicants (* indicates experimentalists):

Gebhard Schertler
Patricia Edwards
Jade Li

Laboratory of Molecular Biology
MRC Centre
Hills Road
Cambridge
UK

Report:

Introduction

Receptors for many different neurotransmitters and hormones produce their intracellular signalling response through the mediation of guanine-nucleotide binding proteins. The best characterised G-protein coupled receptor is the visual pigment rhodopsin. During our previous experiment on the microfocus beamline we obtained sharper diffraction patterns but were unable to screen for crystals under 50μm. Our aim this time was to test diffraction from microcrystals and to try new cryo conditions.

Report

We tried different cryo conditions with crystals frozen either in liquid nitrogen or liquid ethane. In two of these conditions we obtained much improved diffraction, to better than 4Å in one case. We were also able, for the first time, to compare diffraction patterns using both a 10μm and 30μm beam and to compare crystals from 50μm to 10μm in width. The smaller crystals with the 10μm beam gave the best diffraction patterns. The crystal shown in our figure is approximately 10μm wide and the resulting diffraction pattern was taken using a 10μm beamsize. The scanning movement along the y direction was essential in aligning these smaller microcrystals.

We were able to collect data sets from some of the crystals, however, after a short time beam damage was visible in the higher resolution range. Data analysis of these images indicates a primitive hexagonal lattice with the approximate unit cell constants of a=b=104 c=77Å.

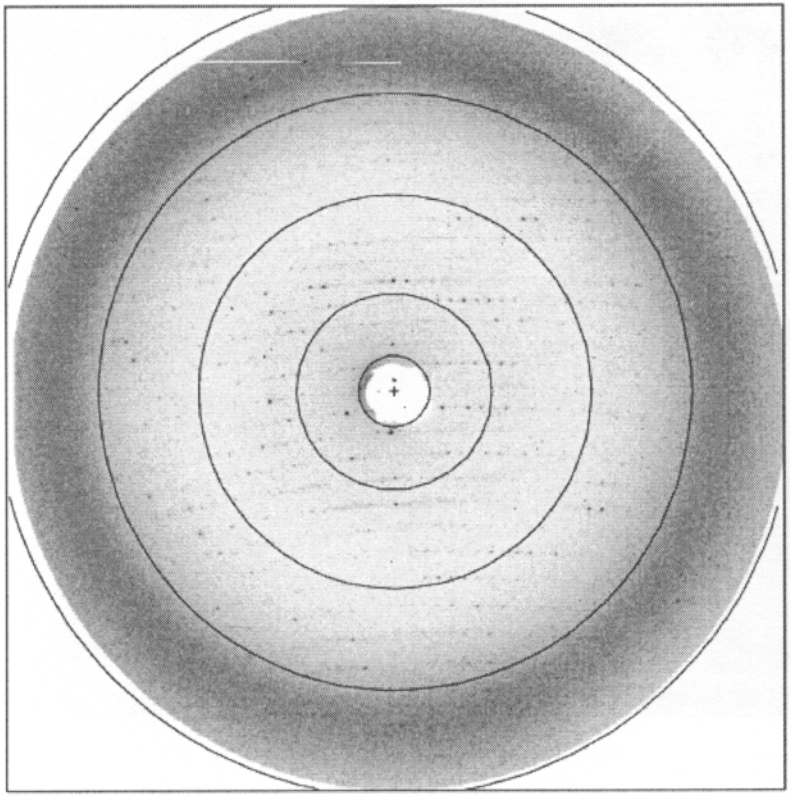
Summary

We were able to check improved cryo conditions, to collect data from crystals down to 10μm in size and obtain partial data sets from these, some diffracting to 4Å resolution. The microfocus beamline was essential in these experiments. Our figure summarises our best findings.

Perspectives

1. Our aim is to repeat these conditions, collect complete data sets and to screen for heavy atom derivatives. In addition to this we have crystals of truncated rhodopsins which might diffract to higher resolution.
2. A device that can scan the cryo-loops in both x and y will improve our chances for data collection.
3. A faster computer at the beamline with MOSFLM installed for data processing would help in assessing diffraction data immediately and for selecting optimal data collection strategy. We will need to check unit cells to make sure we have isomorphous crystals and to monitor beam damage during data collection. More memory available to store data before tape backup would be helpful because last time data collection had to be interrupted to generate storage space.

Rhodopsin at ESRF Microfocus Beamline



Bovine Rhodopsin at 110 K
λ=0.0782 nm
10 μm beam
1^0 rotation
20 sec exposure
MAR CCD
G. Schertler et al.,
LMB Cambridge UK