ESRF	**Experiment title:** Structure determination of a mammalian phosphoinositide 3-kinase	**Experiment number:** LS-981
Beamline: ID14-3	**Date of experiment:** from: 15/2/98 to: 15/2/98	**Date of report:** 18/8/98
Shifts: 1	**Local contact(s):** Wilhelm Burmeister	*Received at ESRF:* 28 AOUT 1998

Names and affiliations of applicants (* indicates experimentalists):

Roger L. Williams*
Edward H. Walker*

MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB22QH
UK

Report:

Our objective is to determine the three-dimensional structure of a mammalian phosphoinositide 3-kinase (PI3K). In addition to being a key component in phospholipid signalling, the role of this enzyme in inflammation, diabetes, cancer and autoimmune diseases has made it the target of extensive, world-wide pharmaceutical development. The enzyme phosphorylates phosphoinositides in the cell membranes to yield 3-OH phosphorylated products (PtdIns3P, PtdIns(3,4)P,, PtdIns(3,4,5)P,) that act as membrane-resident second messengers that are indispensable for insulin-stimulated glucose uptake, suppression of apoptosis, growth factor-dependent mitogenesis, agonist-induced membrane ruffling, vesicular trafficking, and phosphorylation of protein kinase B.

During the very brief time we were allocated at ID14-3 for testing the diffraction characteristics of our PI3K crystals, we collected data to 3.2 Å resolution for the 110 kDa enzyme. This contrasts with weak diffraction to 8 Å using a rotating anode X-ray source. The monoclinic crystals grow with unit cell dimensions a=145 Å, b=68 Å, c=107 Å and C2 symmetry.

The principal reason for the weak diffraction is that the crystals are extremely small. Typical dimensions for the crystals that were the subject of our initial investigation at ID1 4-3 were 80 x 10 x 10 μm^3. In light of the excellent diffraction that we were able to obtain for these small crystals, we concentrated our crystallisation efforts on this crystal form. Using somewhat larger crystals that we have more recently grown, we have identified at low resolution, with other X-ray sources, a set of suitable heavy atom derivatives of these crystals. These new larger native and derivative crystals will be the subject of future higher resolution data collection at ESRF.