ESRF	**Experiment title:** STRUCTURE OF PHOSDUCIN/TRANSDUCIN βγ COMPLEX	**Experiment number:** LS-391
Beamline: D2AM	**Date of Experiment:** from: 07/4/96 14/6/96 to: 09/4/96 15/6/96	**Date of Report:** 10/3/97
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Report:

Activation of heterotrimeric G-proteins by heptahelical transmembrane receptors is a common theme at the start of many signal transduction pathways. One such pathway, in the vertebrate retina, starts when rhodopsin absorbs a photon causing transducin Ga to bind GTP and dissociate from transducin **βγ.** Transducin **Gα-GTP** then activates cGMP phosphodiesterase leading to a drop in the concentration of cGMP closing certain cation channels. The intrinsic hydrolysis of transducin **Gα-GTP to Gα–GDP** leads **Gα-GTP** to reassociate with transducin **βγ** ready for the cycle to start again. Phosducin regulates this cycle. Upon exposure to light phosducin is dephosphorylated on Ser 73 and subsequentley binds to transducin **βγ,** this prevents transducin **βγ** from binding to **Gα-**GDP, and so the inactive **Gα-GDP** cannot be reactivated by the receptor.

In collaboration with **Prof. Yee-Kin** Ho (University of Illinois, Chicago), one of us (A.L.) has developed a purification and crystallization procedure for the phosducin/transducin **βγ** complex from bovine retina. Although the complex is quite tight phosducin is an unstable protein, which is rapidly degraded if isolated from the complex, The inherent instability of the phosducin as well as post-translational **medications, such as a farnesyl moiety at the C-terminal end of the transducin γ-subunit** make the complex difficult to handle and unstable. After extensive crystallization trials the quality of crystals has improved markedly and crystal growth conditions were found **which allowed crystals to be frozen. However, heavy** atom trials both on a home source

and using the synchrotron radiation source at Daresbury have not, to date, yielded a good heavy atom derivative (even crystals grown in the presence of heavy atoms appeared not to have high levels of substitution).

The table, below, summarizes the data collection statistics of the best three datasets collected on beam-line D2AM (several other datasets were collected on phosducinltransducin βγ crystals on D2AM but they were not of such good quality).

Dataset name	Cell dimensions	Resolution	R_{merge} (%)
Native	76.1 87.9 98.7Å	2.8Å	8.0
Gold	75.9 89.3 108.5Å	3.0Å	10.0
Platinum	76.1 89.1 106.4Å	3.0Å	6.9

The overall completness of the dataset varies from 85.5 to 93.2%. The data collected on D2AM are of much better quality than data collected elswhere and made the structure determination possible (e.g. the best dataset collected elsewhere, had data to only 3.5Å and an Rmerge of 10.7% - from five crystals at room temperature at Daresbury). [Note an attempt to collect a MAD dataset on a crystal grown in the presence of Irridium was not successful; during alignment the crystal appeared to move out of the cryo-stream and stopped diffracting.]

The determination of the crystal structures of two heterotrimeric G proteins, $Gi\alpha_1\beta_1\gamma_2$ (Wall et al., 1995) and transducin, $G_{t}\alpha\beta\gamma$ (Lambright et al., 1996) reported in December 1995/January 1996, meant that we were able to locate the transducin βγ part (some 411 ammo acids) of the phosducin/transducin βγ complex by molecular replacement (using coordinates from Walls et al., 1995). Cross-crystal averaging between the closely related cells allowed us to identify the backbone of the phosducin subunit (245 ammo acids), however, the electron density maps were difficult to interpret for the phosducin subunit. Refinement of the bovine retinal phosducin/transducin βγ complex is continuing and the structure currently has an R (Rfree) of 30.5% (35.5%) and includes 572 ammo acids The absence of a substantial part of the structure from the starting molecular replacement model has made the refinement time consuming and difficult (only some 330 amino acids of the transducin βγ subunits were well defined in the molecular replacement solution). Unfortunately, from our point of view, another group, whom we did not know were working on the structure reported the crystal structure of a phosducin/ transducin βγ complex in November 1996 (Gaudet et al., 1996), they had used recombinant material and did not have the c-terminal modification on the γ subunit.

To conclude, the use of beamline D2AM et the ESRF enabled us to collect 2.8Å data on crystals of bovine retinal phosducin/transducin βγ. Unfortunatley the inherent instabilty of the crystals/complex was problematic in obtaining good derivative and or MAD data. We hope to report our crystal structure in the near future.

Gaudet, R., Bohm, A., Sigler,'P.B. (19%) Cell, 87,577-588. Crystal structure at 2.4A of the complex of transducin βγ and its regulator, phosducin.

Wall, MA., Coleman, D.E., Lee, E., Iniguez-Lluhi, J.A., Posner, B.A., Gilman, A. G,, and Sprang, S.R. (1995) Cell, 83, 1047- 1058. The structure of the G protein heterotrimer $Gi\alpha1\beta1\gamma2$.

Lambright, D.G., Sondek,J., Bohm, A., Skiba, N.P. Hamm, H.E., and Sigler, P.B.,(1996) nature, 379, 311-319. The 2.0Å crystal structure of a heterotrimeric G protein.