# EUROPEAN SYNCHROTRON RADIATION FACILITY

**ESRF User Office**
BP 220, F-38043 GRENOBLE CEDEX, France
Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr


# Application for beam time at ESRF – Experimental Method

This document should consist of a maximum of two A4 pages with a minimal font size of 12 pt.

## Aims of the experiment and scientific background

Background
Biosynthesis of the neurotransmitters dopamine, noradrenaline, and adrenaline from the amino acid phenylalanine proceeds by a pathway which involves 5 metalloenzymes. Genetic defects in the first of these, Phenylalanine hydroxylase (PAH) are responsible for "phenylketonuria", also known as "Føllings syndrome". The first two enzymes, PAH and Tryrosine hydroxylase (TH) have been the subject of a major research project lead by Professor Torgeir Flatmark in the University of Bergen.

Human PAH is a 3-domain enzyme containing regulatory, catalytic and tetramerisation domains. Using data collected at BM01A (SNBL) Heidi Erlandsen from this laboratory solved the structure of the catalytic domain (Nature Structural Biology, 1997,4, 9995-1000) and of a series of complexes with Catecholamines.

The total reaction for hydroxylation of Phenylanine (F) to Tyrosine (Y) by PAH is :

$$PAH(FeII) + F + BH_4 + O_2 = PAH(FeII) + Y + BH_2 + H_2O$$

Where $BH_4$ is the unstable reduced cofactor Tetrahydrobiopterin and $BH_2$ is the oxidised cofactor dihydrobiopterin. There are thus *three substrates* and *three products* for the reaction.

Until recently all crystallographic studies have been carried out on the stable but biologically inactive Fe(III) form of the enzyme. However recent experiments carried out at SNBL by us have lead to the crystals structure of the active PAH(FeII) form, both as free enzyme (1.7Å) and in complex (1.5Å) with the*reduced* cofactor $BH_4$. The latter structure is so well resolved that the presence of $BH_4$ rather than $BH_2$ is confirmed by its non-planarity. Furthermore both structures indicate ligand changes around the metal ion which are almost certainly associated with its catalytic function. These are almost certainly indicate that the metal ion is in the Fe(II) state. An article[1] describing these results has been published recently.

Subsequent data collection at SNBL in 2001 lead to the identification at 2.5 Å of the binding site for the slow-turnover substrate Thienylalanine and also showed movement of $BH_4$, a change in the coordination pattern for the Fe(II)-ion and a large and completely unexpected refolding of a 15 residue loop exterior to the active site. This results in an 18Å displacement of a Tyrosine residue bringing it into the active site. These results have been submitted for publication[2].

Studies have thus identified binding sites for $BH_4$ and the substrate amino acid and have lead to a proposed Oxygen binding site

Data collected during the February 2002 SNBL-allocation have lead to the structure of a second Fe(II)/BH4/aminoacid complex. In this case the amino acid is Norleucine. The new structure, confirms previous results, including refolding of the external loop *but the resolution of the structure has improved to 2.0 Å.*

PAH is known to bind Nitric oxide (NO) and there is evidence (J.Biol Chem, 1995, 270,30582-30544 and refs. therein) that this occupies the catalytic oxygen binding site. Preliminary experiments with NO were unsuccessful but new experiments are planned, as are new runs with Carbon monoxide and azide.

Full length Phenylalanine hydroxylase is a three-domain protein with Regulatory, Catalytic and Tetramerisation domains. All work in Tromso has been carried out on the Catalytic domain but we are now attempting to crystallise the larger Regulatory + Catalytic domain protein with and without co-factor and substrates under the anaerobic conditions which have been so successful with the catalytic domain alone. Solution of one or more of these will provide valuable information about regulation of the enzyme's very rapid turnover.

**Why SNBL**?
Part of the planned work is intended to identify the binding site of a diatomic molecule in the neighbourhood of the catalytic metal ion. This will require data to the highest possible resolution and thus synchrotron radiation. Furthermore long experience has shown that we consistently obtain data to at least 0.5 Å higher resolution than in our home laboratory.

**The proposal thus concerns** :

1) Data collection for reduced PAH after treatment with NO with and without $BH_4$
2) Data collection for ternary complexes after treatment with CO..
3) Possible data collections for the native or ligand-bound Regulatory + Catalytic domain of the enzyme

## Experimental method

Data collection at 100K using the MAR 345 image plate at SNBL.

## Results expected

Identification of the binding site for di-oxygen.

Clarification of the reaction mechanism

Better understanding of how the kinetics of the enzyme are regulated.

## References (latest, there are many more)

1) O.A. Andersen, Torgeir Flatmark and Edward Hough (2001)High resolution crystal structures of the catalytic domain of human phenylalanine hydroxylase in its catalytically active Fe(II) and binary complex with tetrahydrobiopterin.*J. Mol Biol.* **314**,279-291).

2) O.A Andersen, Torgeir Flatmark and Edward Hough (2002) Crystal structure of the ternary complex of the catalytic domain of human phenylalanine hysdroxylase with tetrahydrobiopterin ans 3-(2-thienyl)-L-alanine, and its impliocations for the mechaniosm of catalysis and substrate activation. J.Mol Biol, (2002) Manuscript submitted