ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1946
Beamline: 14-1	**Date of experiment**: from: 21-Apr-2001 to: 23-Apr-2001	**Date of report**: 22.8.2001
Shifts:	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Michael Weyand*, Dr. Nicolas Thomä*, Dr. Roman Fedorov*,

Tryptophan Synthase

The bifunctional enzyme tryptophan synthase (TRPS) is a paradigm for substrate channelling. The α- and β-subunits of TRPS are arranged in a linear αββα form and catalyze the last two steps in the biosynthesis of tryptophan. In the α-subunit indole glycerole phosphate (IGP) is cleaved; subsequently indole is channeled through a 25 Å long tunnel to the β-active site where it is condensed with serine that has been activated by a pyridoxal phosphate cofactor. The two reactions remain in phase by an extensive network (including a Na^+ ion) of allosteric interactions that are mediated by a rigid but movable domain of the β-subunit. We tried again to determine the structure of the quinonoid intermediate of the β-reaction TRPS. This time we made the Cs complexes at pH 6.5, 7.5 and 8.5. Due to the extreme light-senistivity of the quinonoid complex we cannot use a microspectrophotometer to test whether the crystals contain the intermediate. The refinement of the structures is in progress.

HSV-thymidine kinase:

Herpes Simplex Virus Thymidine Kinase (HSV-TK) is a bifunctional nucleoside monophosphate kinase that catalysis the reversible phosphoryl transfer reactions: ATP + thymidin <--> ADP + TMP and ATP + TMP <--> ADP + TDP. In order to investigate the catalytic mechanism we crystallized HSV-TK with the bisubstrate inbihibitor TP4A (mimiking the first reaction) from PEG. The dataset is 98.4 % complete to 2.6 Å resolution. The nucleotide is clearly visible, but the resolution is not high enough to identify the Mg^{2+} ion unambigously.

LOV domain

Phototropin, a major blue-light receptor for phototropism, exhibits blue-light-dependent autophosphorylation and contains two LOV (**L**ight, **O**xygen or **V**oltage) domains and a Ser/Thr kinase domain. The LOV domains belong to the PAS superfamily of sensor proteins. They contain a noncovalently bound FMN and exhibit a reversible photocycle that allows them to function as signalling switch. We crystallized the LOV1 domain of *Chlamydomonas* and collected a native dataset to 2.6 Å resolution. Molecular replacement was used to establish the spacegroup ($P6_522$) and to determine the structure.

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme specific inhibitors. We crystallized the nNOS heme domain from rat in an orthorombic spacegroup. Molecular replacement attemps with the 3.5 Å resolution dataset were not successful.

Bx1

Bx1 is a plant protein used in the defense mechanism against insects. It has very high sequence similarity to the alpha-subunit of tryptophan synthase and catalyzes the cleavage of indole glycerol phosphate to indole and glyceraldehyde phosphate. A native dataset had been collected by Klaus Scheffzek, EMBL. Since we could not determine the structure by molecular replacement, we collected datasets of a Pt and Hg derivative. Unfortunately, the structure could not be solved with these data.

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1946
Beamline: ID14-4	**Date of experiment**: from: 7-May-2001 to: 8-May-2001	**Date of report**: 22.8.2001
Shifts: 3	**Local contact(s)**: Dr. Gordon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Axel Scheidig, Dr. Nicolas Thomä*

Report:

Proteins involved in nuclear transport

The small GTPase Ran determines the directionality of nuclear transport via its interactions with its effectors, namely nuclear transport factors (importins) and Ran binding proteins (RanBPs). Recently, a number of structures have been solved which have tremendously increased our knowledge about the molecular details of nuclear transport. Two different import receptor structuress were solved, namely importin-β and transportin, and their complexes with Ran gave insight into formation and dissociation of nuclear import complexes, including the cargo. However, nothing is known about the structure of exportins. Since their interaction with Ran is assumed to be different from the importin interaction, it would greatly help the elucidation of nuclear export if a structure of an exportin would be known.

We crystallized a fragment of the tRNA export receptor exportin-t and could collect a 99% complete dataset to 2.5 Å resolution. We are currently preparing the selenomethionine-derivative of the protein for structure solution using MAD.

Myosin-S2 fragment

Myosin is a key motor protein in muscle contraction. Recently a family of myosin-binding proteins MyBP, MyBP-C and MyBP-H have been identified that seem to regulate myosin function. MyBP-C binds to the N-terminal 126 residues of the myosin rod S2 segment. Human mutations have been described in both MyBP-C and the 126 amino acid long region of S2, which result in familial hypertrophic cardiomyopathy (FHC). We crystallised the S2 fragment carrying the E924K FHC-mutation in P1 (a=33.5, b=41.8, c=111.5, a=90.1, ß=95.4, γ=109.6) and the wildtype in C2221 (c=40.4, b=49.1,c=372.3). We collected a native dataset of the wildtype to 3.0 Å resolution, a dataset of a Pt derivative of the triclinic E924K mutant, and datasets at the peak and inflection wavelengths of a Se-Met derivative of the mutant. The triclinic crystals are highly non-isomorphous, the self-rotation function changes even within

datasets belonging to the MAD experiment. It is therefore not too surprising that we could not solve the phase problem.

Bx1

Bx1 is a plant protein used in the defense mechanism against insects. It has very high sequence similarity to the alpha-subunit of tryptophan synthase and catalyzes the cleavage of indole glycerol phosphate to indole and glyceraldehyde phosphate. Since we could not determine the structure by molecular replacement, we collected a dataset of a samarium derivative. The structure could not be solved.

OxyC

The heme protein OxyC belongs to the cytochrome P450 superfamily. It is involved in the biosynthesis of the medicinally important antibiotic vancomycin, which is derived from a linear heptapeptide. OxyC catalyzes one of the ring formations. After a lot of testing of different crystal forms we identified crystals with monoclinic symmetry that diffract to high resolution. We collected a 1.7 Å resolution dataset. The structure determination by molecular replacement is underway.

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1946
Beamline: ID14-4	**Date of experiment**: from: 08-Jun-2001 to:10-Jun-2001	**Date of report**:
Shifts: 6	**Local contact(s)** Dr. Hassan BELRHALI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists)

Dr. Ilme Schlichting, Dr. Eva Wolf, Dr. Ingrid Vetter, Dr. Balaji Prakash*, Dr. Michael Weyand*, Dr. Axel J. Scheidig*, Dr. Roman Fedorov*, Holger Rehman*

Report:
The following projects were investigated:

Rap guanine-nucleotide exchange factor directly activated by cAMP (Epac II)
We have solved the structure of the 45kda regulatory domain of Epac II, which contains two cAMP-binding domains and a DEP domain for membrane targeting. Initial strutcure determination by SeMet-MAD phasing was done in space group P212121. Problems occuring during the refinement against a 2.5Å dataset suggested the actual space group to be P1 with P212121 pseudosymmetry. Here we collected a 97.7% complete P1 low resolution data set to be merged with a previously collected 84.4% complete 2.5 Å P1-data set, which was incomplete at low resolution due to overloads.

Dynamin
Dynamin is a 100kDa GTPase which, by assembling into spirals around the necks of clathrin coated vesicles, promotes receptor mediated endocytosis and synaptic vesicle recycling. We have obtained needle like crystals of the the N-terminal half of Dynamin comprising the GTPase domain (in presence and absence of nucleotides) and the middle domain, which belong to space group P2. The crystals were not measurable at the home source, but at ID14-4 we were able to collect a 3.2 Å native data set.

ß-catenin
β-catenin is a protein containing an ARM-repeat domain which mediates its binding to APC. Its function is the transport of a transcription factor into the nucleus which activates gene transcription as the last step in the Wnt-signalling pathway. This pathway leads to a certain kind of colon cancer if permanently activated. Since β-catenin is an essential part of this pathway, we would like to investigate its ligand binding properties to find the determinants of APC-binding to catenin. A loop between two Arm-repeats seems to change its conformation upon ligand binding, but its position might also be dependent on ionic strength, pH or

presence of other small molecules. Therefore, we are co-crystallizing β-catenin with different ligands under different conditions and collect high-resolution datasets to determine the conformation of the crucial loop. The final goal would be an interruption of the β-catenin interaction with the other proteins.
On ID14-4, we could collect two datasets with different soak conditions of 2.4 and 2.5 Å resolution, respectively, both 100% complete. In the structures, the loop can be seen in two conformations, and no ligand was bound, contrary to our expectations. This result prompted us to investigate the determinants of the loop conformation in more detail.
At the home source the crystals diffract to only 3.5 Å which is not sufficient for our purposes, so the synchrotron is essential for these data collections.

Myosin-S2 fragment

Myosin is a key motor protein in muscle contraction. Recently a family of myosin-binding proteins MyBP, MyBP-C and MyBP-H have been identified that seem to regulate myosin function. MyBP-C binds to the N-terminal 126 residues of the myosin rod S2 segment. Human mutations have been described in both MyBP-C and the 126 amino acid long region of S2, which result in familial hypertrophic cardiomyopathy (FHC). We crystallised the S2 fragment carrying the E924K FHC-mutation in P1 (a=33.5, b=41.8, c=111.5, a=90.1, ß=95.4, γ=109.6) and collected datasets of crystals derivatized with samarium, EMP, and Pt. In the latter case two datasets were collected, at the peak and inflection wavelengths, respectively. We could not solve the phase problem.

LOV domain

Phototropin, a major blue-light receptor for phototropism, exhibits blue-light-dependent autophosphorylation and contains two LOV (Light, Oxygen or Voltage) domains and a Ser/Thr kinase domain. The LOV domains belong to the PAS superfamily of sensor proteins. They contain a noncovalently bound FMN and exhibit a reversible photocycle that allows them to function as signalling switch. We crystallized the LOV1 domain of *Chlamydomonas* and collected datasets of native and reduced crystals to 2.3 and 2.2 Å resolution, respectively.

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme specific inhibitors. We crystallized the nNOS heme domain from rat in a new C-centered orthorombic spacegroup. The structure could be determined by molecular replacement using the 2.6 Å resolution dataset.

Cytochrome P450cam

P450cam is a heme containing mono-oxygenase that splits O_2 to hydroxylate its substrate camphor to 5-exo-hydroxy-camphor with the concomitant production of a water molecule. Some aspects of the reaction mechanism of this important class of enzymes are still poorly

understood. The mechanistically important double mutant D251N-T252A is unstable and we could only obtain very small crystals (5 x 5 x 30 µm). We collected datasets of a reduced and an oxygenated crystal to 2.0 and 2. 1 Å resolution, respectively. The structures explain the basis for the catalytic characteristics of the double mutant. The manuscript is in preparation.

Bx1

Bx1 is a plant protein used in the defense mechanism against insects. It has very high sequence similarity to the alpha-subunit of tryptophan synthase (TRPS) and catalyzes the cleavage of indole glycerol phosphate to indole and glyceraldehyde phosphate. Interestingly, the kinetics of the reaction catalyzed by Bx1 and TRPS is rather different. A structure comparison should help to understand this finding. Since the structure determination by MIR did not work, we decided to switch to MAD. The datasets collected at peak, inflection and high energy remote wavelengths of selen could be interpreted by Solve. The structure has been refined to 2.0 Å resolution. Publication is hampered by the fact that the active site is completely destroyed by crystal contacts.

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1946
Beamline: ID14-3/1	**Date of experiment**: from: 23-Jun-2001 to: 24-Jun-2001	**Date of report**:
Shifts: 3	**Local contact(s)**: Dr. Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Axel Scheidig, Dr. Roman Fedorov*, Victor Kulik*

Report:

ID14-3:

General remarks:

ID 14,EH3 was in extremely bad shape, the intensity was so low that Hassan Belrhali advised us not to use it. We managed to collect three datasets of strongly diffracting crystals. Fortunately, we got the night shift at ID14-EH1 from Klaus Scheffzek, EMBL.

ß-catenin

β-catenin is a protein containing an ARM-repeat domain which mediates its binding to APC. Its function is the transport of a transcription factor into the nucleus which activates gene transcription as the last step in the Wnt-signalling pathway. This pathway leads to a certain kind of colon cancer if permanently activated. Since β-catenin is an essential part of this pathway, we would like to investigate its ligand binding properties to find the determinants of APC-binding to catenin. A loop between two Arm-repeats seems to change its conformation upon ligand binding, but its position might also be dependent on ionic strength, pH or presence of other small molecules. Therefore, we are co-crystallizing β-catenin with different ligands under different conditions and collect high-resolution datasets to determine the conformation of the crucial loop. The final goal would be an interruption of the β-catenin interaction with the other proteins.

We collected two datasets of 2.9Å resolution, both 99% complete. The mother liquor was changed to PEG instead of urea as in the first data set, and here we could see the loop in a completely "closed" position. This result gave interesting insights into the determinants of the loop conformation which we can exploit for ligand binding studies. At the home source the crystals diffract to only 3.5 Å which is not sufficient for our purposes, so the synchrotron is essential for these data collections.

Myosin-S2 fragment

Myosin is a key motor protein in muscle contraction. Recently a family of myosin-binding proteins MyBP, MyBP-C and MyBP-H have been identified that seem to regulate myosin function. MyBP-C binds to the N-terminal 126 residues of the myosin rod S2 segment. Human mutations have been described in both MyBP-C and the 126 amino acid long region of S2, which result in familial hypertrophic cardiomyopathy (FHC). We crystallised the S2 fragment carrying the E924K FHC-mutation in P1 (a=33.5, b=41.8, c=111.5, a=90.1, ß=95.4, γ=109.6) and collected a datasets of a crystal derivatized with uranyl-acetate. We could not solve the phase problem, most likely due to non-isomorphism developing during X-ray exposure.

ID14-1:

General remarks:

ID 14,EH3 was in extremely bad shape, the intensity was so low that Hassan Belrhali advised us not to use it. We managed to collect three datasets of strongly diffracting crystals. Fortunately, we got the night shift at ID14-EH1 from Klaus Scheffzek, EMBL:

RabGGTase:REP-1 complex

The Ypt/Rab proteins are members of the Ras superfamily of small GTP-binding proteins and have been implicated in the mechanisms by which transport vesicles identify and fuse with their target compartment. Crucial for their functionality is the posttranslational prenylation of C-terminal cysteins by the action of the Rab geranylgeranyltransferase. We have crystallized the active RabGGTase:REP-1 complex and performed the first preliminary X-ray diffraction experiments. The crystals are extremely thin and only the high brilliant synchrotron beam enabled the first characterization of these crystals. We were able to collect a nearly complete data set up to 3.4 Å resolution in the space group $P2_1$. Using molecular replacement techniques we were able to obtain a first CA-trace of both proteins. In the meantime we could improve the crystals and propose to collect diffraction data to higher resolution.

Myosin-S2 fragment

Myosin is a key motor protein in muscle contraction. Recently a family of myosin-binding proteins MyBP, MyBP-C and MyBP-H have been identified that seem to regulate myosin function. MyBP-C binds to the N-terminal 126 residues of the myosin rod S2 segment. Human mutations have been described in both MyBP-C and the 126 amino acid long region of S2, which result in familial hypertrophic cardiomyopathy (FHC). We crystallised the S2 fragment carrying the E924K FHC-mutation in P1 (a=33.5, b=41.8, c=111.5, a=90.1, ß=95.4, γ=109.6) and collected a dataset of a crystal derivatized with thiomersal. We could not solve the phase problem, most likely due to non-isomorphism developing during X-ray exposure.

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme

specific inhibitors. We crystallized the nNOS heme domain from rat in an orthorombic spacegroup. We collected datasets of the native and CN-complexed nNOS to 2.0 Å and 2.45 Å resolution, respectively. Refinement of the native dataset is completed, but there are problems with the cyanide complex.